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THE BIOLOGY OF TROPHOBLAST—SOMA ANTAGONISM AND TESTICULAR TUMORS

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(Received for publication on August 1, 1947)

In an extensive report by Friedman and Moore on Tumors of the Testes (17) the authors are led to ask the fundamental question whether or not "a common oncologic principle underlies all new growths or whether testicular neoplasms are truly unique." That the question is being asked at this time by clinician and pathologist is most important because it has become all but settled for experimental oncologists (34), some biochemists (20), and the cancer-minded embryologist (4). In harmony with this growing conviction the thesis has been proposed, the evidence for which is detailed elsewhere, (3, 4, 21, 29) that a common onocologic principle does exist and is none other than the trophoblast, a tissue which possesses to a remarkable degree every important characteristic associated with cancer (15).

The unusually complete report by Friedman and Moore (17) further substantiates the trophoblastic origin of cancer by showing, once more, that in testicular tumors the morphology of the trophoblast can be effectively masked. Regardless of polymorphic modifications all testicular tumors appear to contain trophoblast as a common denominator responsible for their malignancy (12, 17, 18).

It is interesting to ask why a reciprocal relationship between trophoblast and soma is not more readily discerned in the face of mounting evidence for the trophoblastic origin of cancer and its precocious antagonism to somatic tissue (3, 21, 29). It has been demonstrated by Maximov (30) that trophoblast grown *in vitro* with its accompanying embryonic tissue, which it completely destroys and absorbs in culture, sometimes contains various types of cells found in malignant tumors. It is strange that for all its obvious character the antagonism between cancer and its host is a relationship which, compared with other properties, is very seldom stressed. And yet it is

the fundamental character of this antagonism which may very well provide the key enabling us to fit cancer snugly within the biological scheme of things.

If this antagonism is something which does not appear *de novo,* as an adventitious pathological phenomenon, does it have a counterpart in normal biological processes? Is cancer a unique expression of it? Surely it can be said that the kind of antagonism which cancer exhibits toward its host resembles the antagonism between the pregnancy trophoblast and the soma—be it embryonic or maternal (15, 37). It is also strikingly similar to certain antithetic relations which exist in lower forms between the asexual and sexual generations. This is well illustrated by the echinoderms in which the sexual generation destroys and absorbs the asexual larva, while the axis of the former develops perpendicular to that of the latter (36).

Thus we are confronted with the rather insistent proposition that an understanding of the cancer problem may require that we recognize in a malignant growth the existence of a tissue: (1) which bears a fundamentally antithetic relationship to its host, such as is exhibited by the pregnancy trophoblast, (2) which does not arise or develop from the soma, (3) which cannot give rise to embryonic or somatic elements, and (4) which does not possess the embryonic potencies. Cancer appears to be a tissue which develops in *complete physiological autonomy* of its host (this is true also for the pregnancy trophoblast (51), which does not and cannot arise from somatic elements or give rise to them. This state of affairs, which appears strange only from the point of view of everyday routine pathology, becomes clear when we view cancer as an expression of what is commonly known in zoology as metagenesis: the alternation of an asexual with a sexual generation (5, 21, 44, 49). Although more commonly associated with lower forms, metagenesis is recognized in vertebrates including mammals (44, 45, 49). It has been well studied in armadillos, (47) which produce identical twins through the evocation of totipotent germ cells within the trophoblastic blastocyst. This type of asexual reproduction is normal for man, and occasionally also results in twinning through evocation within the trophoblast of more than one totipotent germ cell (21). Thus in vertebrates, and notably in mammals, the relationship between the asexual and sexual generations, which alternate in the life cycle, results in marked antithesis

because of their close juxtaposition (37), for the nutritional requirements of the embryo require the asexual generation trophoblast (5) to behave as a particularly aggressive parasite (5, 6) during the first two months of pregnancy.

A correlation of the apparently confused mass of data, constantly augmented by careful and critical observations, would depend therefore upon recognition of the phylogenetic concept of vertebrate metagenesis (44, 49). Otherwise the admirable report by Friedman and Moore (17) cannot do full justice to a very interesting and important series of observations.

Thus, there may be nothing new and unusual in the kind of antagonism displayed by cancer which so often results in riot and destruction to its host. This antagonism resembles the normal expression of a certain phase of the life cycle (5, 6, 36, 37). In vertebrates, and particularly in the placental mammals, the asexual generation trophoblast is extremely aggressive in the early phase of pregnancy and becomes relatively passive in the later stages. Indeed, in some rodents the trophoblast disappears altogether before the young are born (23). In the primates, however, the last irreducible vestige of the trophoblast has to be expelled mechanically with the placenta. It may truly be said that if cancer were not fundamentally the analogue of a normal and ordinary phenomenon all metazoan life on earth would be impossible.

The abnormality of cancer is most likely not the cancer itself, but the time and place it appears. With apparent design to divorce trophoblast from cancer in causal relation, Ewing wrote that although trophoblast possesses properties identical with cancer the former is not malignant because it is kept under control (15). The essence of the problem is, precisely, the question of control. We see in trophoblast a normal tissue performing normal functions in pregnancy which, nevertheless, has to be kept *under control* if it is not to destroy embryo, or mother, or both. What sort of tissue is this? And is it not clear that its relation to any host can only be described as antagonistic? Indeed it is because this reciprocal relationship has been recognized by embryologists that the term conceptus has been introduced to describe the embryo *together with* its antithetic trophoblast. The latter is segregated during very early cleavage (22, 26, 44, 51) from a zygote, or activated gamete: a diploid totipotent cell, or germ

cell, having completed gametogenesis. In mammals the trophoblast, or its anlage, appears to be segregated during the first cleavage (22), from its sister cell which is once more a totipotent germ cell. From then on the trophoblast cells and the totipotent germ cells, sometimes called embryo-forming cells, *go their separate way*. As Streeter expresses it "the one can never become the other" (51). If an embryo is to result, it is only through differentiation of a totipotent germ cell and not from a trophoblast cell which belongs to another generation and possesses no potencies for embryonic or somatic development (51).

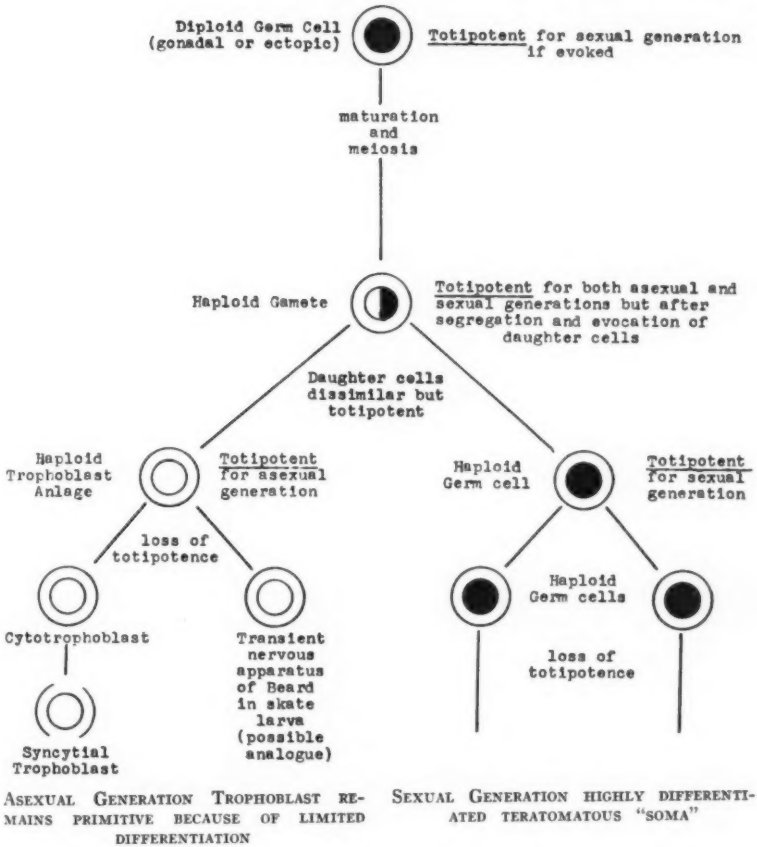
Since a correct interpretation of the findings of Friedman and Moore (17) gives added weight to the trophoblastic origin of cancer a number of quotations from their report have been selected for discussion.

On page 593 the following statement appears: "The cell of origin of embryonic carcinomas and teratoid tumors has the capacity to form both somatic and trophoblastic tissues, as might be expected of a primitive embryonic cell." A carcinoma acquires its specific architecture as a result of tissue reactivity on the part of the surrounding soma, stimulated into more or less active growth by the presence of trophoblast (12). The latter arises from a totipotent cell *which has undergone gametogenesis* and the gamete is thus capable, after activation, of giving rise to trophoblast totipotent cells, as well as other non-trophoblastic totipotent germ cells. It is important to keep in mind that throughout the development from a totipotent germ cell, through gametogenesis and gamete formation—with the latter's capacity to give rise to the two generations of the life cycle, through beginning of cleavage and the segregation of the cell of origin of the asexual generation (trophoblast) from the cell of origin of the sexual generation (embryo), there is and there can be no loss in potency. Since a totipotent cell can only arise from another totipotent cell, totipotency is maintained throughout gametogenesis and ends only with beginning of differentiation. This is illustrated in the following diagram.

The diagram can also be considered to represent the ontogeny of the pregnancy conceptus, to which, of course, the teratomas are related. However, two *haploid* germ cells are figured in the evocation of the embryonic elements of the teratoma. These two germ cells are

DIAGRAM

ontogeny of malignant teratomas


Diploid Germ Cell (gonadal or ectopic)
Totipotent for sexual generation if evoked
maturation and meiosis
Haploid Gamete
Totipotent for both asexual and sexual generations but after segregation and evocation of daughter cells
Daughter cells dissimilar but totipotent
Haploid Trophoblast Anlage
Totipotent for asexual generation
loss of totipotence
Cytotrophoblast
Syncytial Trophoblast
Transient nervous apparatus of Beard in skate larva (possible analogue)
Haploid Germ cell
Totipotent for sexual generation
Haploid Germ cells
loss of totipotence

ASEXUAL GENERATION TROPHOBLAST REMAINS PRIMITIVE BECAUSE OF LIMITED DIFFERENTIATION

SEXUAL GENERATION HIGHLY DIFFERENTIATED TERATOMATOUS "SOMA"

symbolic of any number which may be evoked. In normal pregnancy, barring twinning, only one *diploid* germ cell is evoked into definitive embryogenesis. Left out of the diagram on the trophoblast side is the capacity demonstrated by Hertig (25), for that tissue to differen-

tiate a primitive asexual generation mesoderm and primitive angioblasts, specifically from primitive cytotrophoblast, to form the early circulation of chorionic villi. The fate of these asexual generation structures has so far not been determined. But, in view of the identity of trophoblast with the asexual generation, it is safe to assume that the primitive chorionic vascular bed is eventually replaced by the vascular development of the sexual generation embryo. Moreover, it seems fairly clear that the formation of this primitive vascularity of the villi occurs independently of the vascularization of the embryonic stalk and at a different time (25). This replacement would be analogous to what occurs during the development of the definitive sexual generation of the echinoderms upon their asexual generation larva, which is a complete organism with skeleton, nervous system, enteric, vascular, connective and other tissues. The complete disappearance of the trophoblast during gestation in some rodents, as a result of which the chorionic vessels of the embryo's circulation bathe directly in the maternal blood (23), provides a degree of support for replacement, the probability being that these remaining vessels are not trophoblastic but embryonic.

Pursuing the analogy we can expect cancer to illustrate on occasion the various types of differentiation of which the trophoblast, or an asexual generation, is capable. Indeed, this might well represent one important result of somatic response to the presence of trophoblast, particularly in sarcomas. For example, somatic organizers, which stimulate differentiation and proliferation of the host's tissues surrounding trophoblast, might be expected to have an organizer effect upon analogous asexual ectopic trophoblast primordia. Somatic response to trophoblast within connective tissues could organize primitive trophoblast to form spindle cells of the asexual generation connective tissue. Such connective tissue is well known in the asexual generation hydroids and again in echinoderm larvae. And it should be recalled that Maximov found such cells, and others as well, in his embryo-trophoblast cultures (30). Of course, it is to be expected that, although morphologically similar, the asexual generation spindle cells in a sarcoma will have different properties from cells of the sexual generation soma's connective tissue. This has been found to be the case in cultures of sarcomatous tissue in which the two types of spindle cells, normal and malignant, were separated. Some of the

properties studied were: malignancy, phagocytic properties, acid production, lysis of different types of coagula and other properties (13). These differences were maintained after continued culture. Thus, the statement by Paget, made in 1870, that cancers are "imitation tissues" (35) takes on added significance and may be explained in terms of morphogenesis.

Tissue destruction of one generation by the other may underlie a part of Ewing's discussion on teratomas (16). He says, "while the parasitic fetus does not give rise to secondary blastomatous processes, the teratoma frequently originates true tumors, benign from the adult elements, malignant from the embryonic structures." Since there can be no antagonism between the host and true embryonic structures which are of the same generation as the adult soma, Ewing's malignant embryonic structures can only mean trophoblast (sometimes called primitive ectoderm), a term which does not differentiate it from the embryo which develops within it.

The following statement by Ewing (16) is even more significant: "An important principle of the growth of teratomas is the tendency of one element, either adult or embryonal, to overgrow and suppress the others." Recall the echinoderms or substitute the term conceptus for teratomas and we have a general description, in terms which are to some extent obsolete, of the trophoblast-soma (embryo and mother) relationship of pregnancy which normally terminates in the suppression of the trophoblast; but which may also terminate in the opposite way. And when Ewing refers to "benign (tumors) from the adult elements" we should recognize in these, embryonic structures which most probably have destroyed their accompanying trophoblast just as the "malignant . . . embryonic structures" of uniform type have destroyed their accompanying true embryonic tissue of the sexual generation. It may be stated in passing that all dermoid cysts are benign and well differentiated, if not oriented, because they have, most likely, previously destroyed their accompanying trophoblast.

The cell giving rise to trophoblast, and of course embryo-forming cells as well, has to be similar to a gamete—not a "sex cell," not a germ cell *per se*, but a gamete. What then becomes of the totipotent embryo-forming cells in malignant tumors? In the case of most carcinomas, for example, it is clear that none is evoked into embryonic tissue. Morphogenetically speaking, we may say that the primary

organizer is absent or ineffective (31). There is no embryonic evocation from a totipotent cell (46).

In the case of embryonal carcinomas and teratoid tumors, the situation is quite different. Here, not only is trophoblast formed after cleavage and segregation of the cellular elements resulting from an activated gamete; but *pari passu* there is definite evocation and differentiation of one or even more embryo-forming totipotent germ cells with a release and manifestation of definite embryonic potencies. There is no well organized tissue formation, however, in teratoid tumors (38). This is most likely due to marked interference with organization and axiation by the infiltrating trophoblast of the asexual generation, which in such tumors prevents normal development of the somatic sexual generation. At the same time the individuation field of the adult soma also tends to inhibit tissue organization in its own analogous generation, growing in the wrong place. This inhibition may even be enhanced because the adult soma and the embryonic teratoid growth may not have the same genetic composition. From carcinomas to teratomas every gradation may, therefore, be expected. From no evocation whatsoever of embryo-forming totipotent cells existing within the trophoblast, through partial or distorted organization of embryonic structures resulting from a resistance against what may be termed morphogenetic trophoblast pressure, but at a cost of a lack of control of tissue organization or individuation fields, to finally a more or less developed embryonic tumor which, as in normal pregnancy, may succeed in destroying its trophoblast and, as a result, becomes benign.

If there is no evocation of embryo-forming cells the tissue reactivity or somatic response comes, not from newly evoked embryonic elements, but from the soma of the host in which the trophoblast develops. Of course both types of response may occur at the same time, adding to the morphological complexity of the tumor. It is therefore necessary to consider the two possible types of tissue reactivity in so-called embryonal carcinomas. The primary embryonic type which, in a general way, may be classed with the teratoid tumors has already been considered. The other way may be brought about by a reactivity to trophoblast within an adult soma, not by *differentiating* pluripotent cells which give the invested trophoblast the architecture of an adenocarcinoma, for example, but by the pluripotent

cells themselves which react by dividing mitotically *without differentiation*. It is possible, moreover, that there is an interference with differentiation thus influencing the pluripotent cells to increase in number. Because of the embryonic character of the undifferentiated pluripotent cells the architecture of the tumor would simulate that of an embryonal carcinoma without involving primary embryonic tissue. It is probably this type phenomenon which leads some pathologists to believe that an embryonal type of tumor in an adult represents a reversion of cell type. The general types of tissue reactivity or somatic response may be summarized and tabulated as follows:

Types of tissue reactivity	Resulting tumor types
(adult refers to any post-natal soma)	
little or none	anaplastic tumors and some chorionepitheliomas
embryonic (from evocation of totipotent germ cell or cells segregated from anlage of invading trophoblast)	embryonal carcinomas and teratoid tumors in which both trophoblast and embryonic "soma" have the same genetic composition.
somatic—embryonic (from non-differentiating primitive pluripotent cells of adult soma)	"embryonal" carcinomas of adult somatic origin. Genetic composition of trophoblast and investing tissue may not be alike.
adult somatic (from differentiating pluripotent cells or functional cells still capable of mitotic division)	carcinomas and sarcomas

The maintenance of tissue individuation in the soma depends upon continued differentiation rates by pluripotent cells. This, in turn, depends upon the maintenance of an adequate reservoir of pluripotent cells which is probably controlled in part by general tissue nutrition. Hence, the increasing tendency may be expected, with ageing, for normally dispersed *totipotent* cells, which in vertebrates play no part in body making, to escape this individuation field pressure and undergo gametogenesis after proper stimulus, finally giving rise to cancer. Moreover, any totipotent embryo-forming cell segregated *pari passu* with trophoblast will not tend to form embryonic structures because the pressure of an individuation field of the host is probably exerted with greater force against the sexual generation than against trophoblast. If this were not true pregnancy in mam-

mals would be difficult or impossible. Once trophoblast is evoked ectopically through gametogenesis it has escaped the soma's individuation field. In other words, gametogenesis outside the gonads does not normally occur, nor does activation of a gamete followed by cleavage normally occur within the gonads. Cleavage should normally occur outside the soma.

Individuation field pressure is most likely mediated chemically. Carcinogenesis, whether by radiation, hydrocarbons, hormones or viruses is likewise chemically mediated. The escape from the tissue's individuation field which carcinogenesis represents, is brought about through the concentration and persistence of the carcinogen. Since the trophoblast possesses no potencies for embryonic development it tends to be immune against the individuation field pressure exerted by the tissue in which the trophoblast develops. The tissue reacts by *mechanically* limiting and interfering with trophoblast growth through changes in volume and direction of growth of its own cells. Thus chemical inhibition of trophoblast probably does not originate *in situ*. Chemical inhibition is most likely produced from a distant site and humorally mediated. Vascularization of a malignant tumor may represent an attempt by the soma, not to feed the tumor but to bring to bear increased amounts of the humorally mediated chemical inhibitor. This phase of the problem will be the subject of a subsequent communication.

It cannot be overemphasized that "a primitive embryonic cell" can only give rise to other embryonic cells; not to trophoblast cells (31, 51). When the primary organizer activates an embryo-forming totipotent cell to differentiate, its fate is sealed. Only embryonic and, eventually, adult soma will result whose progressive development will depend upon the continued expenditure of the potencies residing in the original embryo-forming totipotent cell. No trophoblast, no primary sex cells, and no germ cells can ever result from embryonic or somatic cells (1, 10, 14, 43, 29). Moreover, as development proceeds cellular potencies once expended can never be regained (1, 10, 14). It is to be regretted that this important generalization is consistently ignored by many oncologists.

Returning to Friedman and Moore's report we read on page 584 the following: "The trophoblastic potency of embryonic cells is expressed in the chorionepitheliomas and other trophoblastic tumors

while the complementary potency to form somatic structures is expressed in the teratoid tumors." Embryonic cells do not possess trophoblastic potency, a fact well recognized by embryologists. Moreover, it is fruitless to compare the potencies of cells of different generations. To change from one into the other is morphogenetically impossible.

A similar problem occurs on page 588: "The glands and other differentiated epithelial structures in teratocarcinomas were intimately associated with both carcinomatous epithelium and organized mesenchymal components in such a way that they appeared to have differentiated *in situ* from the carcinomatous cells." Somatic differentiation from carcinomatous cells is clearly incompatible with well established cellular potency relationships discussed before, unless possibly what the authors have in mind, when they refer to carcinomatous cells, is not the malignant component, namely the trophoblast, but the investing soma, the result of tissue reactivity on the part of the *adult* soma to the presence of trophoblast elements. This, of course, would be quite different from newly evoked *embryonic* tissue elements of teratoid character. On the other hand, since adult somatic tissue also possesses a reservoir of cells of relatively high potency which are responsible for regeneration, it might be impossible to know if certain non-trophoblastic structures have an adult somatic, or a teratomatous origin except for the fact that, in testicular tissue, regeneration in man does not readily occur (24).

The following is quoted from page 584: "If a teratoma has no histologically malignant components, the qualified designation 'adult' is justified; the term 'benign' should never be used, because metastasis of testicular tumors which appeared to be only adult teratomas has occurred." This carefully worded statement can be considered along with the following on page 598: "There are several possible interpretations of the malignant behavior of the 'benign' adult teratoma. First, it is possible that the testicular neoplasm was only one of several teratoid tumors which developed multicentrally along the course of the genitourinary tract and that the truly malignant neoplasm was extragenital. Secondly, it is conceivable that adult differentiated teratoid tissue had invaded blood vessels and metastasized as such." Invasion of blood vessels and metastasis can only occur if trophoblast, whether masked or overt, is present in the lesion. The

rare occurrences of transport of somatic tissues, or so-called autotransplants, are not considered. The authors have already shown that trophoblast is evident in many of their tumors. Just as there is a balance between trophoblast and soma in pregnancy, which can be upset, on the one hand, by overgrowth of trophoblast followed by chorionepithelioma or hydatid mole, on the other hand, by the premature destruction of trophoblast resulting in abortion or death of the embryo, so there is often, for a time at least, a balance between trophoblast and somatic tissue in cancer. The extremes are anaplasia on the one hand and complete arrest or possible destruction of the malignant growth on the other hand. Between these two extremes a distribution curve could probably be drawn on the basis of the frequency of occurrence of tumors of various degrees of malignancy, since both anaplastic tumors and those which are spontaneously arrested are infrequent. In other words, *apparent* metastasis or invasion by "adult differentiated teratoid tissue" of embryonic character can occur; but the adult tissue is transported with the malignant trophoblast and is not the primary invader.

Other important aspects are raised on page 593: "The new growths of the male gonad appear exceptional in that they do not reproduce testicular tissue (unless the theory of the tubular nature of seminomas is accepted) [the authors have previously shown that the tubular theory is most likely incorrect. C. G.] while most other neoplasms reflect the architecture of the tissue in which they originate. They express, in distorted fashion, the latent unlimited potencies of the embryonic germ cells from which they arise, while other tumors can make manifest only the limited potencies of their somatic cells of origin. It is important to determine whether a common oncologic principle underlies all new growths or whether the testicular neoplasms (and other teratoid growths) are truly unique." The crux of the situation is that the irreversibility of cellular potencies (1, 9, 14) makes it impossible for somatic cells to give rise to trophoblast. This law, which will aid materially in transforming biology into something approaching an exact science, also points significantly to the common oncologic principle which underlies all malignant growths. So far as testicular tissue is concerned it is a poor example of tissue reactivity and in man is, in fact, ill adapted to regeneration and repair (24). Hence there is a tendency for trophoblast to remain recognizable and

not be masked by tissue reactivity. It explains why heterologous transplants are feasible in testes. And the results of Friedman and Moore's observations illustrate this point well. In the first place, the authors find a relatively high proportion of embryonal carcinomas (19%), which as they point out, are really types of chorionepitheliomas. The invading trophoblast is exposed to tissue response not from the adult testis, but from the embryonic tissues differentiated from one or more totipotent cells segregated from trophoblast after testicular gametogenesis. As a result, and in a manner suggestive of the formation of chorionic villi, the trophoblast tends not to be masked in these instances. In the second place, lack of tissue reactivity of the testis is shown by the preponderance of embryonal carcinomas, teratocarcinomas and teratomas which altogether account for 60% of all testicular tumors reported (17). Again, it is worthwhile pointing out that embryonic cells do not possess "unlimited potencies" for reasons already discussed; and that germ cells, which are indeed totipotent, and may, in a sense, be said to possess "unlimited potencies" cannot be characterized correctly as embryonic. It is true that they are normally present in embryos and adults, both as genital cells and as extragenital functionless cells which do not take part in body making (26, 51). It is also true, of course, that they constitute the embryo-forming cells wherever there is embryonic evocation. But as totipotent cells they are neither embryonic nor somatic since none of their potencies has as yet been expressed. Moreover, they do not arise from the soma, but are morphologically continuous with the germ cells segregated during early cleavage of the pregnancy conceptus—one of which gave rise to the embryo and adult which contains them in the gonads and elsewhere (2, 7, 16, 21, 27, 28, 48, 50). They do not, therefore, arise from or belong to the sexual generation (10). Nor do they form part of the asexual generation characterized in mammals by the trophoblast. The germ cells are the pivotal cells, which after intracellular changes occurring during gametogenesis and activation normally give rise to trophoblast and simultaneously to more totipotent germ cells. On the other hand, by undergoing differential mitosis (11) they may develop embryowise and thus lose for all time both their immortality and their capacity to give rise again to the asexual generation. After differentiation, totipotent cells, now no longer totipotent are incapable of gametogenesis.

The totipotent germ cells are now definitely known to comprise the unending stream of cells which, as John Beard expressed it, "link the remote past with the distant future." They manifest their potentialities by becoming now trophoblast and germ cells, now soma, by different morphogenetic processes and in a divergent manner. They constitute the pivotal cells of the metagenetic life cycle.

The statement in Friedman and Moore that "tumors can make manifest only the limited potencies of their somatic cells of origin" could conceivably represent a novel way to explain the relatively low malignancy of some tumors. However, it would be necessary to postulate that a somatic cell of low potency, or no potency at all, can dedifferentiate into a cell of high potency belonging, in addition, to another generation, an idea altogether incompatible with modern findings (1, 9, 14, 31).

It is worthwhile stressing the point that the significance of important morphologic findings such as characterize the report of Friedman and Moore can remain equivocal from the pathologist's traditional viewpoint, but they can be clearly understood if we keep in mind the fundamentally autonomous and antithetic character of the trophoblast and the soma, and recognize that again in the words of Streeter, "the one can never become the other."

Friedman and Moore have added further evidence to the growing realization that carcinomas and other tumors of the testis may contain trophoblast even when it is not morphologically recognizable as such (8, 17, 18). Testicular tumors are probably not unique in this respect, for the presence of ectopically dispersed germ cells which alone can give rise to trophoblast after first undergoing gametogenesis *in situ* is attested by much direct as well as indirect evidence (2, 7, 16, 21, 27, 28, 48, 50). There is, therefore, nothing radical or surprising in the concept which has been advanced; namely, that all malignant tumors contain trophoblast as the malignant component (4, 21, 29). The difficulty appears to lie with the general conditioning to, and preoccupation with, ideas which are biologically untenable.

In view of the much greater tissue reactivity in the extragenital soma in man, masking of the trophoblast outside of the gonads should be the rule rather than the exception.

A discussion on testicular tumors would be incomplete if advantage were not taken of some remarkable observations made before the war

by Peyron, Limousin and others. The relationship to metagenesis implied by the authors is revealed by their studies. They claim to have "demonstrated polyembryony, comparable to that shown in Hymenoptera . . . in testicular tumors, with multiple tissues, in male subjects. The teratological development of an egg in the male genital gland of man has been anticipated from theory, since placental tissue has been demonstrated in testicular embryomas. However the first stage of the teratological blastoderm giving rise to the tumor had not yet been discovered. We have identified the precocious stage of the embryonic bud in seven of our tumors (or about 1/10 of those studied in serial sections of various samples). The buds, generally very numerous, from several hundred to several thousand, can be reconstructed during the entire development of the tumor. They are formed by two juxtaposed cellular ridges: one amnio-ectodermal, appearing precociously—the other appearing later . . . represents primitive endoblast. . . ." (43). "It is understandable that buds are absent in most tumors. Serial sections are indispensable to show them, as well as the distinction between trophoblast and mesenchyme at their periphery. These embryomas thus representing prolonged growth of young blastocysts in a male medium provide an object for study much more favorable than the ovarian embryomas or even the normal ovary. In the latter Leo Loeb was the first to demonstrate parthenogenetic complexes but they are very rare, almost always found singly, degenerated or arrested at the blastomere or morula stage. On the contrary in our embryomas the buds, sometimes present in extraordinarily large numbers show the greatest variation in size" (43). Unlike the testis the ovary is well adapted to control any development of the mature ovum. For this reason malignant embryomas are rare in the ovary. "Invasion and multiplication in blood vessels occur, of buds having both ectoblastic and endoblastic potentialities. One embolus contained ten buds of about the same size" (41). "Some show three buds in the shape of lobes each with a rudimentary endoblastic vesicle (figure 1). New buds arise from the undifferentiated matrix. The buds have a temporary existence and are reconstituted about as fast as they disappear both in the primary tumor as well as in the metastases. About several hundred thousand buds must have been formed during the evolution of the tumor (two years at least from clinical signs)" (42).

FIGURE I
(*adapted from Peyron*)

THREE EMBRYONIC BUDS WITH SINGLE AMNION DEVELOPING WITHIN TROPHOBLASTIC BLASTOCYST (Blastocyst not shown)

"In conclusion our tumors confirm that the blastoderm (teratological) before exhibiting an irregular neoplastic proliferation must have been subjected to an organizer influence by an axially oriented region in the case where the embryoma was axially oriented and in the second case" (sacrococcygeal parasitic twin—C. G.) "by a regu-

lar germinal segregation followed by consecutively occurring phenomena, migration of gonoblasts, formation of gonadal nidus, development of urogenital connections and the definitive kidney. . . ." (40).

The formation is demonstrated of two kinds of vesicles, which have a different origin: the neoplastic blastocyst which contains trophoblast, and the purely embryonic bud, many of which may be evoked within a single trophoblastic blastocyst. The embryonic buds themselves are of course benign although they invade blood vessels. The authors recognize from their observations the formation of blastocysts from eggs and evocation of embryonic buds within blastocysts, and the fundamental analogy of embryonic buds with sacrococcygeal teratomas described as parasitic twins, from a "regular germinal segregation." Moreover they even describe the presence of germ cells in malignant testicular embryomas (39) and in sacrococcygeal teratomas; their migration within these teratomas and the eventual formation of normal testes(40). Their differentiation between sacrococcygeal polyembryonic twin tumors which are benign, and ovarian and testicular malignant teratomas is significant. The authors point out that the latter types "represent a new generation," perhaps also a new genetic constitution, only attainable through gametogenesis and perhaps also meiosis of the segregated germ cells whether they occur in the primary tumor or within metastatic blastocysts or embryonic buds.

One criticism can be made in the authors' interpretation of their findings. They include all observed proliferations in the general class of polyembryony and suggest an analogy with polyembryony in Hymenoptera and armadillos. This is incorrect, for in polyembryony, as the word implies, there is evocation of more than one embryo within a *single* blastocyst (45). In man, polyembryony occurs during formation of identical twins, or the evocation of more than one totipotent germ cell within a single chorion. Most of the vesicles described, and all neoplastic ones, would be analogous to fraternal twins in man where each twin has its own chorion and is formed from a separate fertilized gamete. In other words, those vesicles which are neoplastic represent a continuous process of gametogenesis of germ cells. Otherwise, new trophoblasts would not be formed. However, the resulting gametes are evoked to undergo cleavage. Whether homologous sperms are involved was not discussed.

At the end of one report there is a discussion note by Caullery: "Teratological polyembryony just demonstrated by Peyron and Limousin seems to me from a purely zoological point of view to have its nearest analogy in the order of normal phenomena in the asexual proliferation of the vesicular cysts of certain cestodes particularly Taenia echinococcus" (43). The analogy with polyembryony holds for the intrablastocystic embryonic buds but, strictly speaking, not for the neoplastic blastocyst buds themselves. On the other hand Taenia echinococcus alveolaris offers an exceedingly interesting and unique example of the antagonism between the asexual generation of a species in one phylum (Platyhelminthes) and the somatic or sexual generation of a species of a distant phylum (Chordata). In Taenia echinococcus two types of development usually occur within the parent and daughter hydatid cysts: Formation of definitive embryonic scolices and formation of new daughter cysts. The formation of scolices is a polyembryonic phenomenon representing the evocation of totipotent germ cells situated within the cyst wall, which is the asexual generation blastocyst analogous to trophoblast in mammals. Moreover, this cestode blastoderm most likely produces, like trophoblast, a type of somatic response on the part of the human host resulting in the formation around the cyst of a tough somatic fibrous tissue surrounding the cyst capsule. The formation of daughter cysts however is really not polyembryony. Daughter cysts consist of a blastoderm which can only arise after one of the totipotent germ cells within the wall of the parent cyst has undergone gametogenesis. Also each daughter cyst has, probably, a new genetic constitution. The polyembryonic scolices arising within each vesicle without being included in new cysts have the same genetic constitution. If a germ cell undergoes gametogenesis and the genetic constitution of the resulting gamete is changed, so is that of any resulting embryo. In polyembryony all embryos have the same genetic constitution.

It sometimes occurs, though rarely, more particularly in certain geographic locations (33), that there is little, abnormal, or no evocation of embryonic scolices within many of the cysts. The balance between the asexual generation cysts and the sexual generation scolices is apparently disturbed in favor of the asexual generation cyst walls, and the host's heterologous soma is unable to restore this balance. This situation may be followed by a luxuriant proliferation of the

asexual generation cysts to form a complex alveolar tissue which invades the host, metastasizes readily, and may have the consistency of colloid cancer (33). In fact, it was confused with cancer during the 19th century until Virchow demonstrated the tumor's real origin (33). Here again we have an example of the essential antagonism between two generations, possibly augmented by an extreme heterologous relationship. The neoplastic testicular buds are analogous to the numerous vesicles of Taenia echinococcus multilocularis whose numerous asexual generation blastoderm cysts usually contain embryonic scolices, although many cysts do not. In Taenia echinococcus alveolaris the asexual generation develops in a suitable medium but in the absence of its own normally developing sexual generation. The phenomenon is reflected in higher forms by the anidian growths in fish and bird eggs in the absence of embryonic evocation (32), likewise in man by the ectopic pregnancy trophoblast, described by Bonney (8), and which develops without any embryo, spreading and eroding like chorionepithelioma.

It would be a deliberate disregard of phylogenesis, if metagenesis, which is so well known in lower forms, were to be ignored, or the belief upheld that it suddenly ceases in vertebrates, particularly since classic examples of it are found among the lower chordates (45). There is little doubt that the properties manifested by the mammalian trophoblast and the extraembryonic blastoderm of egg-laying vertebrates make these tissues analogous to the asexual generation in lower forms. Viewed in this light little difficulty remains in understanding the markedly antagonistic relationship between the sexual generation soma and the asexual generation trophoblast, either in pregnancy or in cancer.

References

1. Abeloos, M. (1932). La régénération et les problèmes de la morphogenèse. Gauthiers-Villars, Paris. 77-79.
2. Abramovicz, H. 1913. Die Entwicklung der Gonaden Anlage und Entstehung der Gonocyten bei Triton taeniatus (Schneid). *Morph. Jahrb.*, **47**, 593-644.
3. Beard, H. H., Halperin, B., Libert, S. A. 1947. Effect of Intraperitoneal Injection of Malignant Urine Extracts in Normal and Hypophysectomized Rats. *Science*, **105**, 475-476.
4. Beard, J. 1902. Embryological Aspects and Etiology of Carcinoma. *Lancet*, Lond., **1**, 1758-1761.
5. ———. 1919. Alternations of Generations and its Bearing upon Cancer. *Dublin J. M. Sc.*, **147**, 217-225.

6. ————. 1897. The Span of Gestation and the Cause of Birth. A Study of the Critical Period and its Effects in Mammalia. Jena: G. Fischer, 22-24.

7. Beccari, N. 1922. Studi sulla prima origine delle cellule genitali nei Vertebrati. II: Richerche nella Salamandrina perspicillata. *Arch. Ital. di Anat. e di Embryol.*, **18**, 29-95.

8. Bonney, V. 1943. Division of Zygote. Producing Trophoblast only. *J. Obst. Gyn. Brit. Empire*, **50**, 217-218.

9. Bounoure, L. 1940. Continuité germinale et reproduction agame. Gauthiers-Villars, Paris, pp. 62-65.

10. ————. 1939. L'Origine des cellules reproductrices et le problème de la lignée germinale. Gauthiers-Villars, Paris, pp. 110-135.

11. ————. Pp. 196-200.

12. Brewer, J. I. 1932. Chorionic Gonadotropin in the Diagnosis of Testicular Tumors. *Arch. Path.*, **41**, 580-591.

13. Ephrussi, B. 1932. La culture des tissus. Gauthiers-Villars, Paris, pp. 44-54.

14. ————. 116-132.

15. Ewing, J. 1940. Neoplastic Diseases. A Treatise on Tumors. Fourth Edition. W. B. Saunders Co., Philadelphia and London, p. 88.

16. ————. P. 1046-1047.

17. Friedman, Major N. B., Moore, R. A. 1946. Tumors of the Testis. A Report on 922 cases. *The Military Surgeon*, **99**, 573-593.

18. Gill, A. J., Caldwell, G. T., Goforth, T. L. 1945. Choriocarcinoma of the Testicle. *Amer. J. Med. Sciences*, **210**, 745-751.

19. Glass, R. L., Culbertson, C. S. 1946. Teratoma of Pineal Gland with Choriocarcinoma and Rhabdomyosarcoma. *Arch. Path.*, **41**, 552-554.

20. Greenstein, J. P. 1947. Biochemistry of Cancer. Academic Press, New York. 367 pp.

21. Gurchot, C., Krebs Jr., E. T., Krebs, E. T. 1947. Growth of Human Trophoblast in Eye of Rabbit. Its Relationship to the Origin of Cancer. *Surg., Gynec. Obstet.*, **84**, 301-312.

22. Hamilton, W. J., Boyd, J. D., Mossman, H. W. 1945. Human Embryology. Williams & Wilkins, Baltimore. 32 pp.

23. ————. Pp. 336-344.

24. Henke, F., Lubarsch, O. 1931. Handbuch der Speziellen pathologischen Anatomie und Histologie. Vol. 6. Part 3. Julius Springer, Berlin. Pp. 627-629.

25. Hertig, A. T. 1931. Angiogenesis in the Early Human Chorion and in the Primary Placenta of the Macaque Monkey. *Contrib. Embryol. Carnegie Institute*, **25**, 37-81.

26. Heuser, C. H., Streeter, G. L. 1929. *Contrib. Embryol. Carnegie Institute*, **20**, 1-28.

27. Humphrey, R. R. 1925. The Primordial Germ Cells of Hemidactylum and Other Amphibia. *J. Morph. and Physiol.*, **41**, 1-43.

28. King, H. 1908. The Oogenesis of Bufo lentiginosus. *J. Morph.*, **19**, 439-468.

29. Krebs Jr., E. T., Gurchot, C. 1946. Trophoblast Elements in Cancer. *Science*, **104**, 302.

30. Maximov, A. 1924. Tissue Cultures of Young Mammalian Embryos. *Contrib. Embryol. Carnegie Institute*, **16**, 47-107.

31. Needham, J. 1942. Biochemistry and Morphogenesis. University Press, Cambridge. Pp. 101, 110-115, 505-506.

32. ————. Pp. 223, 258-259, 507, 327.

33. NEVEU-LEMAIRE, N. 1936. Traité d'helmintologie médicale et vétérinaire. Vigot Frères, Paris. Pp. 628-635, 641-650.

34. OBERLING, C. 1944. The Riddle of Cancer (translation by Woglom, W. H.). Yale University Press, New Haven. P. 152.

35. PAGET, SIR JAMES. 1870. Lectures on Surgical Pathology. Third Edition. Longmans, Green & Co., London. Pp. 382-387.

36. PARKER, T. J., HASWELL, W. A. 1940. A Textbook of Zoology. Sixth Edition. Macmillan, London. Vol. I. P. 741.

37. PATTEN, B. M. 1946. Human Embryology. Blakiston, Philadelphia and Toronto. Pp. 145-166.

38. PETILLO, D. 1944. A Critical Review of the Pathogenesis of Chorioma Testis and a New Theory. *Urol. and Cutan. Reviews*, **48**, 53-65.

39. PEYRON, A. 1938. Sur la présence des cellules génitales primordiales dans les boutons embryonnaires des embryomes parthénogènètiques chez l'homme. *C. rend. Acad. Sc.*, **206**, 1680.

40. ————. ET BONNARD, B. L. 1938. Sur l'origine gemellaire ou parasitaire des tumeurs à tissus multiples de la région sacrococcygienne. *C. rend. Acad. sc.*, **206**, 1197.

41. ————. ET LIMOUSIN, H. 1936. Sur l'embryonie intravasculaire et les metastases à tissus multiples dans les embryomes du testicule. *C. rend. Acad. sc.*, **203**, 894.

42. ————. 1936. Sur le mode de multiplication des boutons embryonnaires et la presence de blastocystes jumeaux dans les tumeurs à tissus multiples du testicule. *C. rend. Soc. biol.*, **123**, 409.

43. ————. 1936. Sur la polyembryonie dans les tumeurs à tissus multiples du testicule chez l'homme. *C. rend. Acad. sc.*, **203**, 631.

44. RICHARDS, A. 1931. Outline of Comparative Embryology. John Wiley & Sons, Inc., New York. P. 190.

45. ————. Pp. 295-321.

46. ————. P. 187.

47. ————. Pp. 362-366.

48. SCHAPITZ, R. 1912. Die Urgeschlechtzellen von Amblystoma. Ein Beitrag zur Kenntnis der Keimbahn der urodelen Amphibien. *Archiv. mikr. Anat.*, **79 (2)**, 41-78.

49. STOCKARD, C. R. 1921. Developmental Rate and Structural Expression; on Experimental Study of Twins, "Double Monsters" and Single Deformities and the Interactions Among the Embryonic Organs During their Origin and Development. *Am. J. Anat.*, **28**, 115-266.

50. STOWELL, R. E., SACHS, E., RUSSELL, W. J. 1945. Primary Intercranial Chorionepithelioma with Metastases to the Lungs. *Am. J. Path.*, **21**, 787-791.

51. STREETER, G. L. 1938. Advances in Our Knowledge of Early Primate Embryo. *Am. J. Obs. Gynec.*, **36**, 747-749.

52. WITSCHI, E. 1914. Experimentelle Untersuchungen über die Entwicklungsgeschichte der Keimdrüsen von Rana temporaria. *Archiv. mikr. Anat.*, **85 (2)**, 9-113.

Growth, 1947, **11**, 155-175.

PRELIMINARY STUDIES OF ATYPICAL GROWTH IN ONION ROOTS SUBJECTED TO CONTINUOUS APPLIED ELECTRIC CURRENTS OF LOW INTENSITIES*

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Introduction

The existence of electrical potential differences in living cells and tissues has been recognized and studied as a valuable part of physiology for more than a century. Most of the work has been devoted either to the classical muscle-nerve action currents or to the theoretical problem of the nature and origin of the electrical potential. As a result, only slight attention has been paid to the possible role of electrical energy in cell dynamics. Is the electricity performing a useful and vital function or is it a mere by-product, the "smoke", of some essential mechanism? Lund (1928) first attempted to answer this question nearly twenty years ago in his theory of electrical cell correlation in which he "proposed that the electrical pattern is intimately related to the morphogenetic processes and polar or vector properties of cell and tissue functions. One function of this electrical field or pattern is to act as a *directive force* in laying down of new structure (i.e. growth), and possibly the orderly transfer of various materials in morphogenesis, ..." (Lund 1946).

The existence of highly complex electrical circuits which correspond, at least in part, to the morphological pattern was described by Lund (1929a, 1929b, 1930, 1931a, 1931b) for the Douglas fir. Similar fields were subsequently demonstrated in the oat coleoptile by Wilks and Lund (1946) and in the mantle of fresh water mussels by Lund and Gunter (1946). These facts, however suggestive, constitute no proof for the theory of electrical cell correlation. Numerous additional experimental facts are now available and it can be seen

*The authors wish to acknowledge with gratitude the technical assistance of Miss Helen Chapman in making some of the slides and drawings and that of Miss Nelly Jane Keffer in making the diagram of the apparatus.

that (1) electrical changes precede and by their orientation predict the direction of tropic response to stimulation by gravity, light and mechanical energy of the oat coleoptile and that these tropisms can be modified by appropriately applied electrical potentials (Schrank 1944, 1945a, 1946a, 1946b, 1946c, 1946d); (2) applied electrical currents of small magnitude and short duration alter the inherent bioelectric potentials of the onion root in such a manner as to suggest a profound modification of the normal potential pattern [Marsh (1930), Lund and Berry (1946), Berry and Hoyt (1943a, 1943b), and Hoyt (1947a, 1947b)]; (3) applied currents of longer duration (1-2 hours) when passed through onion roots in opposition to the inherent polarity reversibly inhibit the rate of elongation and may kill the root when allowed to continue for 24 hours if the IR drop is sufficiently large [Lund, Mahan and Hanszen (1946) and Evers and Lund (1946)]; and (4) applied currents have been shown capable of directing the polarity of regenerating Obelia [Lund (1921) and Barth (1934)], of Fucus eggs (Lund, 1923) of pollen tubes (Marsh and Beams, 1945), and of regenerating nerve fibers in tissue culture (Marsh and Beams, 1946). These facts, considered collectively, along with the work of Burr (1947), offer strong support to the idea that the normal flow of electrical energy through cells and tissues is operating in accordance with the theory of cell correlation. However, the theory predicts that a more profound modification of growth should be possible if the inherent correlating mechanism were altered by suitable experimental technique.

The experiments to be reported in this paper grew out of studies from this laboratory (Berry and Hoyt 1943a and b), previously referred to, on the effects of applied current on onion root potentials. In these experiments the technique made it necessary to remove all but one root from the experimental bulbs. It was observed that enlargements occasionally appeared on a few of these single roots, which upon microscopic examination were found to be regions of active cell division. These regions were in the mature part of the root where mitotic activity does not ordinarily occur. These findings immediately raised the question of whether or not these cells would have divided in any circumstances, or whether the external current altered the correlating mechanism within the root permitting or stimulating the cells to divide. In an effort to answer these questions the

necessity for collaboration between physiologist and cytologist was apparent, and the work was continued with onion roots as the experimental material for a number of reasons: (1) because of its relatively simple tissue organization; (2) because its electrical properties have been extensively studied; and (3) because the normal histology of the root is well-known and variations from the typical pattern are easy to recognize.

Methods

The initial problems to be solved in this investigation were concerned with the standardization of conditions of growth, so that the roots could be grown in the laboratory under environmental conditions as constant and as close to their natural ones as possible, and so that there should be adequate data on standard growth for comparison with the experimental material.

As a nutrient medium, Hoagland's solution (Seifriz 1938) was used, diluted to 25% of its full strength since the full-strength medium had previously been found to have such a high electrical conductivity that the inherent potential of the root was largely short-circuited and the potential measurements were small and inaccurate. Since measurements of the inherent potential are essential to a complete analysis of the effect of an external current, this solution offers a standard nutrient medium for these and future experiments. In the first experiments, a temperature of $25 \pm 0.5^{\circ}$C. was maintained in the nutrient solution by means of a bi-metallic thermostat and a 150 watt heating element, both of which were chromium plated. It was soon apparent that products from the corrosion of the temperature regulating elements were poisoning the roots so that they were removed from the culture vessels and the roots grown at room temperature except in the final series of experiments. In these, they were grown in closed glass tubes, immersed in a water bath kept at $25 \pm 0.5^{\circ}$C., so that they did not come into contact with the medium in which the thermo-regulating units were suspended.

Small, white skinned onion bulbs, obtained from the Barteldes Seed Company, Lawrence, Kansas, were stripped of their dried outer coats and briefly immersed in dilute formalin. They were then inserted in the flanged ends of short glass tubes supported in a plexiglass rack. The rack was placed across glass supports so that the lower ends of

the bulbs were covered by the nutrient solution. The roots were straighter when confined in tubes during their initial growth and measuring and sectioning for cytological examination could therefore be done more accurately. Aeration was accomplished by bubbling air continuously through the nutrient medium by means of a Marco aquarium pump. Under these conditions, when there are no sources of toxic ions, roots first appear from the base of the bulb 24-36 hours after "planting" and grow to an average length of 25-30 mm. within three days, reaching an average length of about 80 mm. in a week's growth. From 20-40 roots originate from each bulb.

A modification of the apparatus used by Lund, Mahan and Hanszen (1946) was employed for passing electric current through the growing roots. A series of U-tubes, connected by horizontal arms and with small cups at their upper ends to hold the bulbs were filled with 25% Hoagland's solution and immersed in a water bath kept at $25 \pm 0.5^{\circ}$C. by a thermoregulator. The apparatus is represented in Figure 1. The terminal horizontal arms of the U-tubes extend beyond the water bath and are attached by one-hole rubber stoppers to electrode cups filled with the nutrient medium. Zinc-zinc sulphate electrodes with long side-arms dip into the cups. In order to prevent the migration of toxic zinc ions into the nutrient solution in which the roots grow, the ends of the horizontal tubes attached to the electrode cups are filled with 2% agar made up in the dilute Hoagland's solution. A continuous drip of fresh solution is maintained in each electrode cup. The overflow from these cups is siphoned off into funnels and run into a waste tank. Another continuous flow of fresh nutrient solution is passed through the U-tubes from the cathodal to the anodal end. The solution drips into a funnel opening at one end of the connecting horizontal tubes and flows out through the outlet tube at the other end and into a funnel connected to the waste tank by rubber tubing.

The 110 volt direct current supply to the Laboratory was used as the source of potential in the electrical experiments. This was connected to the end terminals of a 1000 ohm rheostat and the electrode leads were attached as shown in Figure 1. An RCA Ultrasensitive D.C. meter was placed in series with the circuit through the apparatus and current flow, measured in microamperes, was registered. In order to calculate the IR drop along the root the electrical conduc-

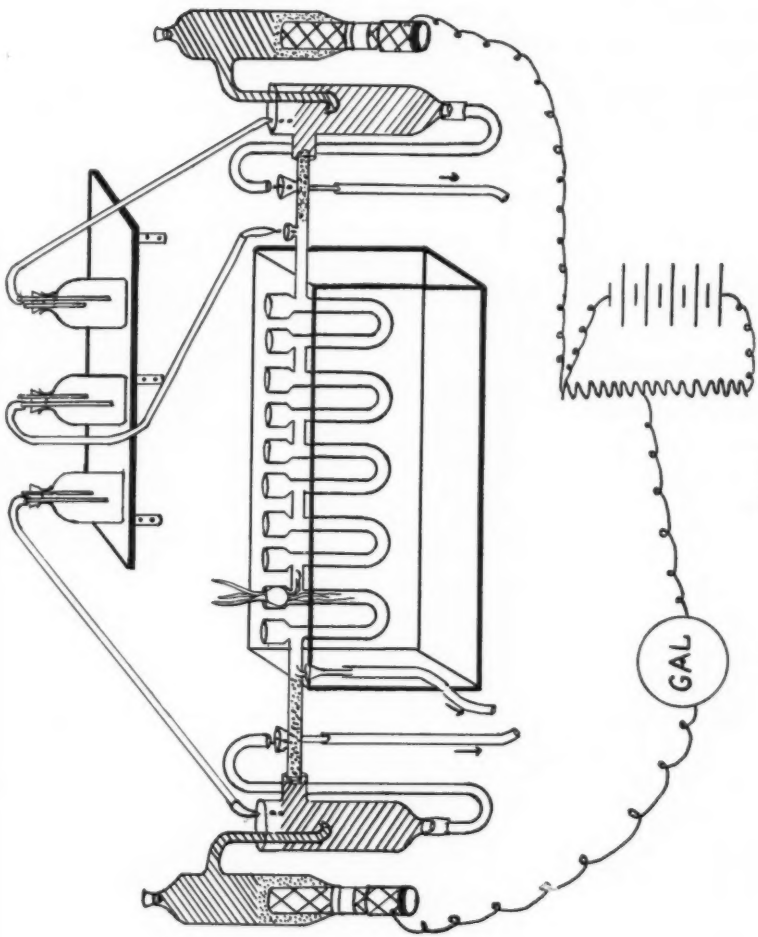


FIGURE 1
DIAGRAM OF APPARATUS USED TO GROW ONION ROOTS IN AN ELECTRIC FIELD. EXPLANATION IN TEXT

tivity of the nutrient medium was measured in the usual way with a bridge circuit and headphones. As Evers (1946) has emphasized, the primary measurement of significance in experiments where current is passed through the medium surrounding living systems is the potential drop per unit length. It is apparent that current alone has little meaning since for a given applied voltage the magnitude of current flow depends upon the conductivity of the solution.

The current was maintained constant to within 3% throughout each experiment. Since some of the bulbs grew out into the horizontal tubes connecting the U-tubes as well as vertically down into them, there was current passing both up and down some of the roots from each bulb. Current passed up in about one-half of the roots from a single bulb and down in about one-half of them. However, the direction of current flow cannot be specifically stated since current undoubtedly passed laterally or diagonally through many of the roots. Two modifications of this apparatus will be made for future experiments: (1) to insure that all the roots grow vertically so that the main passage of current will be down all the roots of one bulb and up all those of another; and (2) to provide better aeration of the culture medium so that the roots, growing as they do close-packed in the tubes, will have an abundant oxygen supply.

In all the experiments reported here, the apparatus was kept in a dark room and all examinations and readings were made in a white light of less than 15 foot candles intensity as measured by a General Electric Exposuremeter. This intensity of light has no apparent effect on the growth of onion roots, for no detectable alteration in growth could be found in control roots grown continuously under such illumination.

Control experiments were conducted simultaneously with the electrical ones by growing roots in an apparatus identical with that through which the current was passed, with the omission of the electrode cups. Both sets of apparatus were mounted in the same water bath and a continuous drip of fresh nutrient solution maintained to both.

Selected roots were fixed in Bouin or in Randolph's Craf (Conn and Darrow 1946), dehydrated through graded alcohols, cleared in xylol and embedded in paraffin. Both longitudinal and transverse serial sections were cut at 7 microns and 10 microns and stained with hema-

toxylin and erythrosin. In some cases, sections of control and experimental roots were mounted on the same slides so that they could be subjected to identical procedure in staining and mounting. Observations were made on both horizontally and vertically grown roots. Nuclease digestion experiments were also conducted on sections of control roots; after digestion the sections were stained with celestine blue and Pyronine-G as described by Sanders (1946). The desoxyribonuclease for these experiments was very kindly furnished by Dr. Maclyn McCarty of the Hospital of the Rockefeller Institute and the ribonuclease by Dr. M. Kunitz of the Rockefeller Institute, Princeton, New Jersey, to both of whom the authors are greatly indebted.

Results

In the normal onion root the actively dividing region, or meristem, extends about 5 mm. along the root. Basal to this region are the cells which are enlarging, principally along the longitudinal axis, and in which the small cytoplasmic vacuoles are coalescing into the large central vacuole characteristic of plant cells in general. Basal to this, and approximately 10 mm. above the tip, the cells are undergoing differentiation. In this region three zones of cells may be recognized: (1) the outer epidermis; (2) the cortex, whose cells form the main mass of the root, and (3) the central cylinder or stele. The inner layer of cortical cells surrounding the stele is known as the endodermis; within this is a layer of cells, the pericycle, and the xylem and phloem tissues, arranged in five alternating groups and enclosing the single central vessel. (Figure 2 and Figure 3).

In many plants the root system consists of primary roots from which, as growth proceeds, secondary or lateral roots originate. It has been shown that in general it is the cells of the pericycle that divide to form the lateral roots, the new cells pushing out through the endodermis, and then through the cortex and epidermis, enlarging and differentiating as they do so. Disintegration of the cortical and epidermal cells appears to take place in the path of the growing lateral root before its emergence (Figure 4). It is generally recognized, however, that onion roots grown in a nutrient solution (or even tap water) rarely form lateral roots. It was our assumption, therefore, that any marked mitotic activity in the pericycle or other cells above the growing point was the response of those cells to some stimulus.

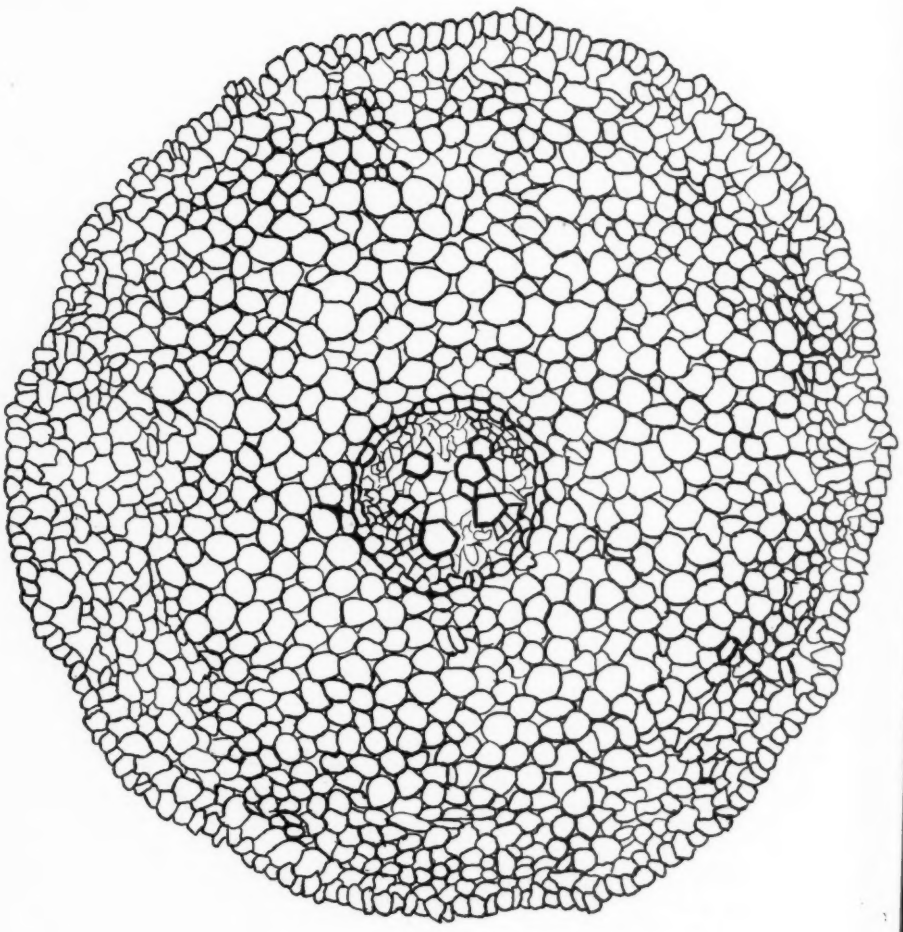


FIGURE 2

CAMERA LUCIDA DRAWING OF CROSS SECTION OF A CONTROL ROOT, 10-15 MM. ABOVE TIP, SHOWING NORMAL DIFFERENTIATION OF TISSUES. BOUIN; HAEMATOXYLIN AND ERYTHROSIN. 80 X

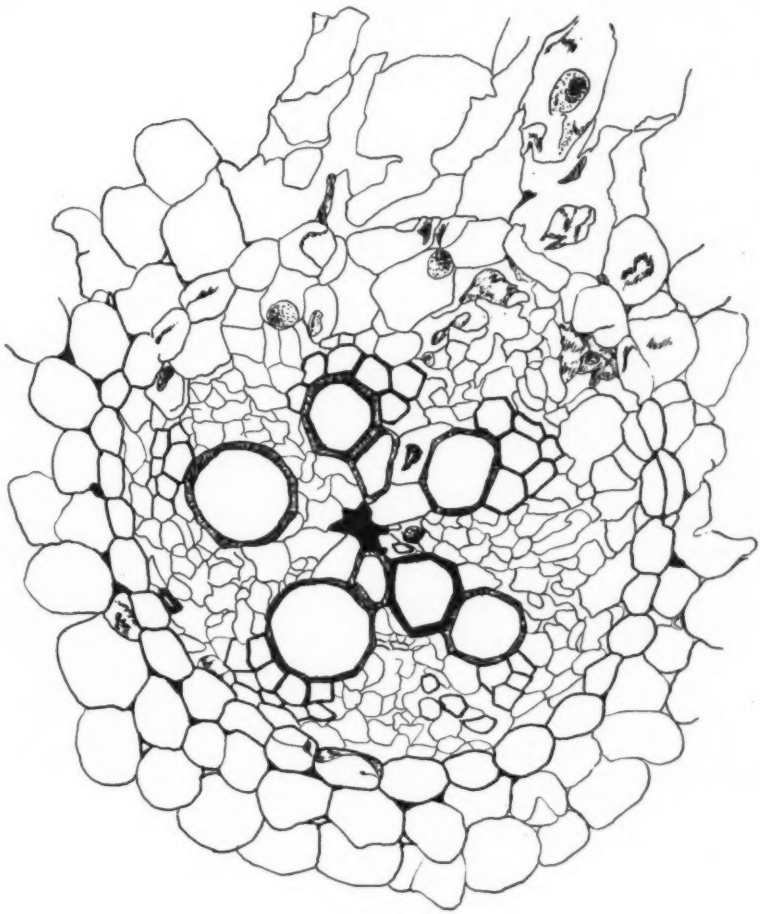


FIGURE 3

CAMERA LUCIDA DRAWING OF CENTRAL CYLINDER OF THE SAME ROOT AS FIG. 2, SEVERAL MILLIMETERS HIGHER UP THE ROOT, SHOWING THE TYPICAL ARRANGEMENT OF XYLEM AND PHLOEM ELEMENTS. LONGITUDINAL SECTIONS OF A LATERAL ROOT A FEW SECTIONS BEYOND THIS; THE ENLARGEMENT AND DESTRUCTION OF THE CORTICAL CELLS AT THE TOP OF THE DRAWING MARK THE LOCATION OF THE ROOT. BOUIN, HAEMATOXYLIN AND ERYTHROSIN. 400 X.

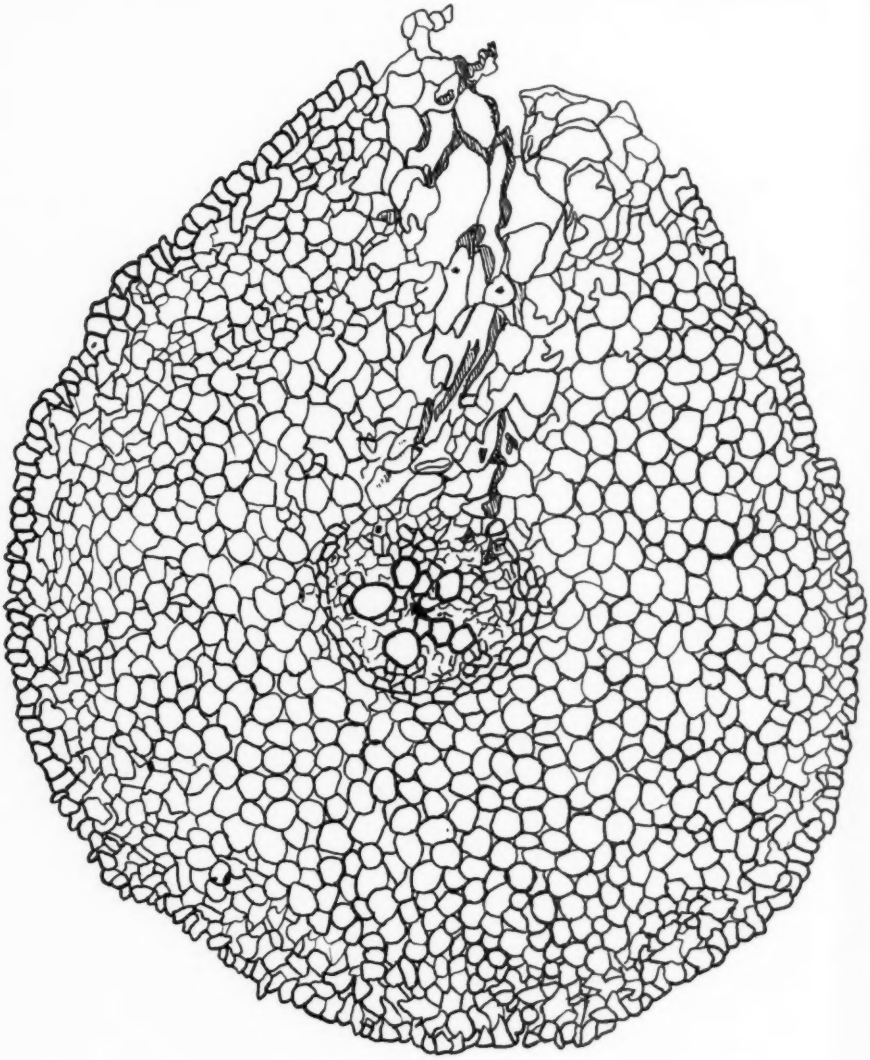


FIGURE 4

CAMERA LUCIDA DRAWING OF A CROSS SECTION OF A CONTROL ROOT WITH A LATERAL ROOT, SHOWING THE DESTRUCTION OF CORTICAL AND EPIDERMAL CELLS IN THE VICINITY OF THE LATERAL ROOT. BOUIN, HAEMATOXYLIN AND ERYTHROSIN. 80 X

The first experiments were made in an attempt to induce enlargements on single roots by the technique used by Berry and Hoyt (1943a and 1943b). These soon revealed that the swellings, which appeared after the passage of current up the root for short periods of time, developed after two or three days into lateral roots. To test whether this was a natural response of the plant to the reduction of surface area of its normal root system or a specific response to the electrical stimulus three sets of ten bulbs each were planted in the nutrient medium, as described above, and their growth watched for 18 days. In the first set, all but one of the roots were removed as soon as they appeared from the base of the bulb, in the second all but two and in the third all were allowed to remain. In the bulbs with but a single root, lateral root primordia appeared on the sixth day after planting; by the eleventh day there were fourteen lateral roots, six of which had already emerged through the epidermis; by the fifteenth day there were 32 lateral roots all through the epidermis and 36 on the eighteenth day. In the second set, where two roots were left on each bulb, lateral root primordia were evident also on the sixth day after planting; there were 23 on the eleventh day, of which 14 were through the epidermis and 42 on the fifteenth day with 37 through the epidermis; no more had appeared on the eighteenth day but all 42 had broken through the epidermis. In the third set, with all roots intact, one root showed a lateral root primordium 11 days after planting; 15 days after planting, four lateral roots had emerged. No more primordia appeared during the remaining three days of the experiment. Taking 35 roots as the average per bulb (see Table II), this represents a ratio of 0 lateral roots to 350 in the first ten days of growth, and of 4 to 350 in an 18 day period. Table I summarizes the results of these

TABLE I

	No. of Bulbs	No. of Roots	Days of Growth	Lateral Roots and Primordia	Ratio Lateral Roots to Total Roots
Set #1	10	10	10	14	14:10
			18	36	36:10
Set #2	10	20	10	23	23:20
			18	42	42:20
Set #3	10	350 (av.)	10	0	0:350
			18	4	4:350

experiments, which were fully confirmed in later ones. In no case, even in the single roots, were lateral root primordia evident in the first five days of growth.

It is therefore apparent that under these conditions of growth lateral roots are very rare if the root system of the bulb is complete. When the root system is reduced to one or two roots only, lateral roots originate in great numbers.

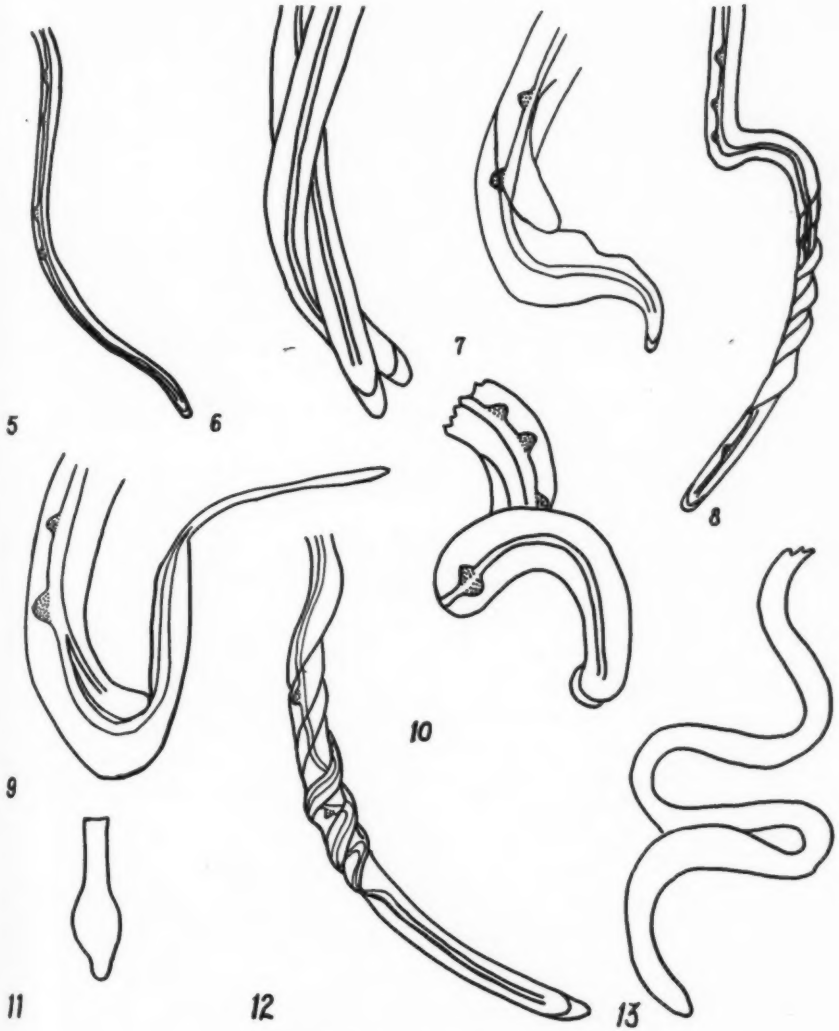
The apparatus shown in Figure 1 was therefore devised so that a current could be applied continuously and simultaneously to all the roots originating from any bulb. Currents from 25 to 200 microamperes corresponding to 4.7 to 37.8 millivolts per millimeter potential drop along the root were used. Table II presents the results of six such experiments, each of which ran for 14 days. In the first experiment, in which 25 microamperes were turned on after two days of growth, there were no more lateral roots than would be expected had no current been applied,—a ratio of 2 to 356 roots, actually less than that of 3:377 of the controls. Potential drops of 9.4, 15.0 and 18.9 millivolts per millimeter (and especially of 15.0) led to the production of numerous lateral roots and their primordia. With a

TABLE II

Date Planted	Date Current on	Current (micro-amperes)	IR Drop (mv./mm.)	Total Roots	Lateral Roots and Primordia	Lateral Roots Through Epidermis	First Appeared
12/24	—	0	—	377	3	2	1/6
1/8	1/11	25	4.7	356	2	1	1/20
1/24	1/27	50	9.4	362	58	15	1/31
2/7	2/10	80	15.0	341	123	69	2/14
3/13	3/13	100	18.9	349	47	12	3/17
3/29	3/29	150	28.3	383	10	1	4/2
4/11	4/11	200	37.8	385	0	0	—

drop of 28.3 millivolts per millimeter, only ten lateral root primordia were visible, and of these only one had broken through the epidermis. There were a number of roots however with gross deformations such as twisting, looping, spiralling, doubling, etc. Figures 5, 6, 7, 8, 9, 10, 11, 12, 13 are sketches of typical abnormalities of these roots.

Local swellings of the roots appeared also after the application of higher intensities of current, which when sectioned were found to be



FIGURES 5, 6, 7, 8, 9, 10, 11, 12, 13
SKETCHES OF GROSS ABNORMALITIES OF ROOTS. 150 MICROAMPERES (IR DROP 28.3 MV./MM.) THROUGH ROOTS CONTINUOUSLY FOR 14 DAYS

FIGURE 11
100 MICROAMPERES (IR DROP 18.9 MV./MM.) THROUGH ROOT CONTINUOUSLY FOR 14 DAYS

due not to division of the cells but to their abnormal vacuolization and swelling in all planes (Figure 14). This occurs in about half the roots when 37.8 mv. per mm. is applied continuously and is accompanied by inhibition of mitotic activity in the root tip, abnormal differentiation and with longer application, general necrosis. The appearance of these swollen cells is similar to that of the cells in neoplasms induced in plants by the application of phytohormones (Gardiner 1940); in the cells of tissue cultures of Jerusalem artichokes grown in media containing indole-acetic acid (Gautheret 1940) and presumably to those of the ovular tumors (unfigured) reported by Blakeslee and Satina (1947) in Datura hybrids. No lateral root primordia appeared under these conditions. It is interesting to note that Lund, Mahan and Hanszen (1946) found that a voltage drop of 37.8 millivolts per millimeter was the threshold for inhibition of growth of the onion root when the time of application of the current was 90 minutes.

The results of these experiments show that continuous applied currents of low intensity, but above a threshold value, produce profound effects upon the growth pattern of the onion root and upon the differentiation of its cells. The development of lateral roots in numbers far in excess of those normally occurring indicates that at certain intensities the current flow results in cell division. This is apparent too in data from the experiments with an IR drop of 18.9 and 28.3 millivolts per millimeter; in both cases lateral root primordia were evident after four days of growth, while in no other experiment did they appear before five days; the sixth day was the earliest on which they were recognizable even in bulbs deprived of all but one root, where there is a pronounced stimulus to lateral root production.

Histological examination of the induced lateral roots and their primordia shows no detectable deviation from the normal in their growth pattern. It is the cells of the pericycle that first divide and from these new cells pushing out through the disintegrating cortex the tissues of the root are differentiated (Figure 15 and Figure 16). The division figures appear normal. It is perhaps of some significance that even in the cases of extreme lateral root production the primordia do not originate at the tip of the primary root, but always some millimeters above it, suggesting that even the pericycle cells have to attain some degree of differentiation before they are capable of responding to a stimulus to divide.

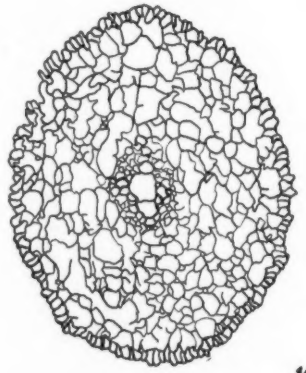


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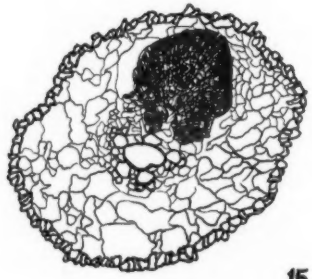
FIGURE 14

CAMERA LUCIDA DRAWING OF EXPERIMENTAL ROOT AFTER 7 DAYS GROWTH. 3 DAYS GROWTH WITHOUT CURRENT; 50 MICROAMPERES (IR DROP 9.4 MV./MM.) CONTINUOUSLY FOR 4 DAYS. BOUIN, HAEMATOXYLIN AND ERYTHROSIN. 80 X



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DRAWING 15

CAMERA LUCIDA OF CROSS SECTION OF AN EXPERIMENTAL ROOT WITH LATERAL ROOT PRIMORDIUM AFTER 10 DAYS GROWTH. 3 DAYS GROWTH WITHOUT CURRENT; 50 MICROAMPERES (IR DROP 9.4 MV./MM.) CONTINUOUSLY FOR 7 DAYS. BOUIN, HAEMATOXYLIN AND ERYTHROSIN. 80 X

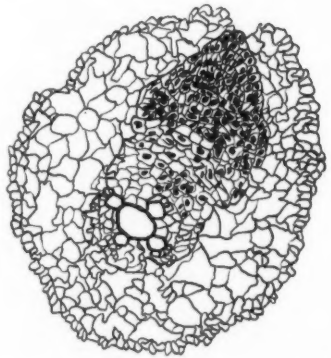

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FIGURE 16

CAMERA LUCIDA DRAWING OF CROSS SECTION OF AN EXPERIMENTAL ROOT AFTER 11 DAYS GROWTH. 3 DAYS WITHOUT CURRENT; 50 MICROAMPERES (IR DROP 9.4 MV./MM.) FOR 8 DAYS. BOUIN; FEULGEN. 80 X.

The most striking histological abnormalities in the experimental roots are the enlargements of the cells of the cortex, the relative thinness of their walls, the distortions of their shapes, the great size of their vacuoles and the disruption of their protoplasts, and the disorganization of the cells of the stele (Figure 17). These abnormalities, as well as the increased mitotic activity, appear to apparently the same extent in all the experimental roots, regardless of the principal direction of current flow.

Discussion

While these experiments are preliminary to a more extensive study of the effects of an external electric field upon the growth of onion roots, they have already shown that an electric current is a measurable and a controllable means of disturbing the normal growth pattern of a tissue and the normal mitotic cycle of a cell. The mechanisms underlying the disturbance are by no means so clear. Several possibilities suggest themselves, all still to be tested. (1) The resemblance of the enlarged cortical cells of the experimental roots to the cells of plants treated with growth hormones suggests a derangement of the growth hormonal system in the roots. This might be either in the synthesis of the hormone so that the end product was unlike that normally produced and consequently different in its action, or it might be the result of its displacement from its customary locations or routes of transport (Schrank), or it might be due to an increased rate of its synthesis. Its displacement or an increase in its production could lead to local accumulations of growth-promoting substance and hence to the local growth, enlargements and types of gross deformations observed. (2) The enlargement of the vacuoles of the cells in the experimental roots suggests that the entrance of water into them is facilitated in some way; the relative thinness of the cell wall supports such a possibility. At least one effect may be directly or indirectly upon the arrangement of micelles in the cellulose lattice. (Bonner and Wildman, 1946). (3) The formation of lateral roots may be an indication of changing polarity in the root. A complete reversal of polarity such as has been found in animal regenerates and in isolated eggs could hardly be expected in a root attached to a bulb, although it might occur in cultured fragments. The reduction in mitotic activity at the tip of the root and its increase in the upper regions

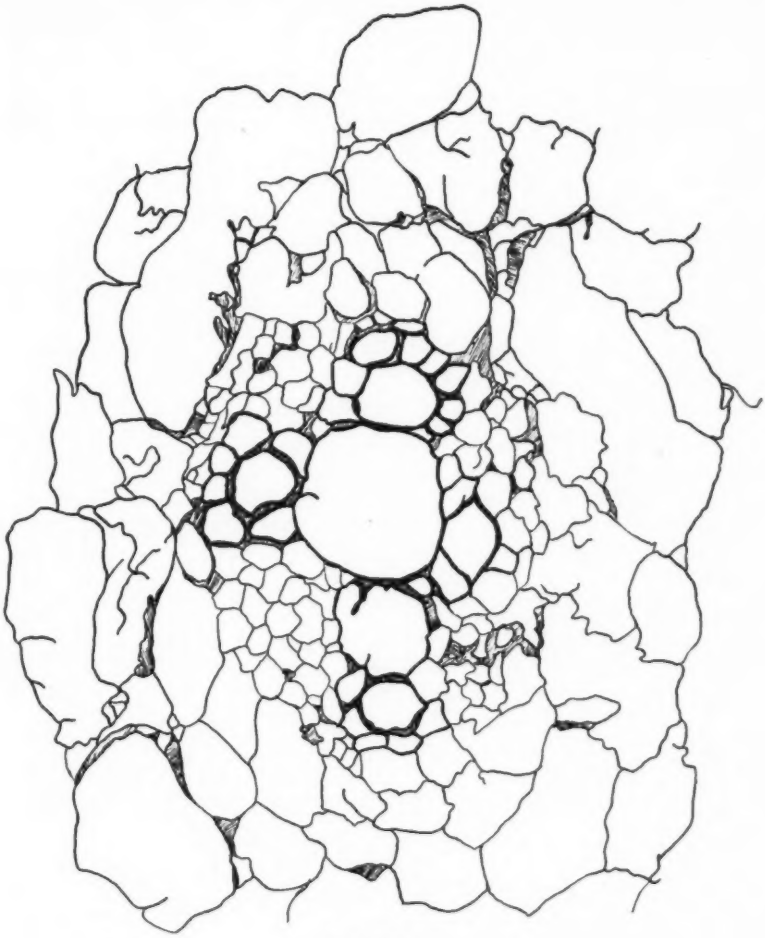

FIGURE 17

CAMERA LUCIDA DRAWING OF CENTRAL CYLINDER OF THE SAME ROOT AS FIG. 14, SHOWING THE DISORIENTATION OF THE DIFFERENTIATED TISSUES. BOUIN, HAEMATOXYLIN AND ERYTHROSIN. 400 X

may be an expression of a shift in its inherent polarity. (4) One or more of the cellular enzyme systems may be altered or inhibited at some stage of its cycle, or stimulated to increased production or activity.

Whatever the mechanism underlying the modifications may prove to be, it is evident that an electric current of low intensity is a precise means of altering the microenvironment of the cells of a system such as the growing onion root. The "directive force" of the inherent potential which Lund postulates is clearly thrown out of line when an external current is caused to flow through the system. This provides a new tool for the study of atypical growth and one that can yield quantitative data in respect to the strength of the stimulus necessary to cause division and abnormal differentiation in cells. It therefore differs from any of the chemical or mechanical agents which have been used as carcinogens. By growing tissue in a controlled external electric field, it should be possible to determine the amount of energy necessary to initiate division in a cell or to stimulate cells to abnormally frequent divisions. Since the time, intensity and space distribution of the stimulus applied can be controlled it should be possible to predict within limits the time at which the change from normal to abnormal occurs and consequently to subject closely related cells to cytological and cytochemical study during the course of their conversion from normal to neoplastic. Such investigations are the object of future research.

Summary

1. White onion bulbs when grown in the dark in 25% Hoagland's solution sprout 20-40 primary roots which grow to an average length of 80 mm. in a week.
2. With a complete root system few lateral roots are produced; approximately 1 in 100 roots in a two week period.
3. If all but one primary root is removed from the bulb, many lateral roots are produced; approximately 140 in 100 roots in 10 days, 360 in 100 in 18 days.
4. If all but two primary roots are removed, the incidence of lateral roots is still high; approximately 120 in 100 roots in 10 days, 210 in 100 in 18 days.
5. When bulbs with their full complement of roots are grown in an electric field, so that currents of low intensity are continually

passing through them, lateral roots are produced in great numbers and earlier than in controls or even in bulbs with but a single primary root. There are also gross and microscopic abnormalities in the primary roots. These deviations from normal growth occur to the same degree in all the roots, regardless of the main direction of current flow, whether up or down the root, under the conditions of these experiments.

6. The greatest number of lateral roots are produced with an IR drop of 15 mv./mm.; there was no increase above the ordinary number with an IR drop of 4.7 mv./mm. Abnormalities were most numerous with an IR drop of 28.3 mv./mm. At 37.8 mv./mm. there was generalized necrosis of the roots.
7. The induced lateral roots had their origin in the pericycle in the ordinary way and the mitotic figures in them were apparently normal.
8. The gross abnormalities in roots grown at the higher intensity of current were local swellings, twisting, looping, spiralling and doubling of the individual roots.
9. The microscopic abnormalities were disorganization of the cells of the central cylinder of the root, extreme vacuolization and enlargement of the cells of the cortex and sometimes their destruction, and thinness of their walls.
10. Possible mechanisms behind this atypical growth are suggested and some lines of future research indicated.

References

1. Barth, L. G. 1934. The effect of constant electric current on the regeneration of certain hydroids. *Physiol, Zool.*, **7**, 340-364.
2. Berry, L. Joe & Hoyt, R. C. 1943a. Polarization and stimulation of the onion root by direct current. *Plant Physiol.*, **18**, 372-396.
3. ———. 1943b. Stimulation of the onion root by alternating current. *Plant Physiol.*, **18**, 570-587.
4. Blakeslee, A. F., & Satina, S. 1947. Ovular tumors associated with hybrid embryos in Datura. *Sci.*, **105**, p. 633.
5. Bonner, J., & Wildman, S. G. 1946. Contributions to the study of auxin physiology. Growth 10 (Supplement), 51-68.
6. Brachet, J. 1933. Recherches sur la synthese de l'acide thymonucleique pendant la developpement de l'oeuf d'Oursin. Archives de biologie, **44**, 519-576.
7. Burr, H. S. 1947. Field theory in biology. *Scientific Monthly*, **64**, 217-225.
8. Conn, H. J., & Darrow, Mary A. 1946. Staining Procedures. *Biotechnical Publications*, Geneva, New York.

9. EVERS, J., & LUND, E. J. 1946. Reversible decrease in rate of growth by an electric current applied to a small region of the root. Pp. 197-217 in: E. J. Lund & collaborators, Bioelectric Fields and Growth, Austin, University of Texas Press, 391 pp.

10. ————. History of the problem of polar bioelectric fields and polar growth under conditions of applied electric fields. Pp. 151-161 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. Austin, University of Texas Press, 391 pp.

11. GARDINER, M. S. 1940. The effect of beta-indole acetic acid upon isolated plant embryos. *Bulletin of the Mount Desert Island Biol. Lab.*, 22-23.

12. GAUTHERET, R. J. 1946. Plant tissue cultures. GROWTH: 10 (Supplement), 21-43.

13. HOYT, R. C. 1947a. Counter-emfs in the onion root. *J. Cell. & Comp. Physiol.*, **29**, 109-130.

14. ————. 1947b. Potential oscillations in the onion root resulting from current flow. *J. Cell. & Comp. Physiol.*, **29**, 131-148.

15. LUND, E. J. 1921. Experimental control of organic polarity by the electric current. 1. Effects of the electric on regenerating internodes of Obelia commissuralis. *J. Exp. Zool.*, **34**, 471-493.

16. ————. 1923. Electrical control of organic polarity in the egg of Fucus. *Bot. Gaz.*, **76**, 288-301.

17. ————. 1928. Relation between continuous bioelectric currents and cell respiration. II. 1. A theory of continuous bioelectric currents and electric polarity of cells. 2. A theory of cell correlation. *J. Exp. Zool.*, **51**, 265-290.

18. ————. 1929a. Electric polarity in the Douglas fir. *Publ. Puget Sound Mar. (biol.) Sta.*, **7**, 1-28.

19. LUND, E. J. 1929b. The relative dominance of growing points in the Douglas fir. *Publ. Puget Sound Mar. (biol.) Sta.*, **7**, 29-37.

20. ————. 1930. Internal distribution of the electric correlation potentials in the Douglas fir. *Publ. Puget Sound Mar. (biol.) Sta.*, **7**, 259-287.

21. ————. 1931a. External polarity potentials in the apex of the Douglas fir before and after mechanical stimulation. *Plant Physiol.*, **6**, 507-517.

22. ————. 1931b. Electric correlation between living cells in cortex and wood in the Douglas fir. *Plant Physiol.*, **6**, 631-652.

23. ————. 1946. P. 284 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

24. ———— & BERRY, L. JOE. 1946. Polarizable and "nonpolarizable" electrochemical systems in the polar cell. Their significance in ion transport and growth. Pp. 161-186 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

25. ———— & GUNTER, G. 1946. Electric polarities in a simple epithelial system. Pp. 15-24 in Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

26. ————, MAHAN, R. I., & HANSZEN, A. H. 1946. Reversible total inhibition of polar growth in the root by an applied current and the nature of this control. Pp. 186-197 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

27. MARSH, G. 1930. The effect of applied electric currents on inherent cellular E.M.F. and its possible significance in cell correlation. *Protoplasma*, **11**, 447-474.

28. MARSH, G., & BEAMS, H. W. 1945. The orientation of pollen tubes of Vinca in the electric current. *J. Cell. & Comp. Physiol.*, **25**, 195-204.

29. ————. 1946. In vitro control of growing chick nerve fibers by applied electric currents. *J. Cell. & Comp. Physiol.*, **27**, 139-157.

30. SANDERS, F. K. 1946. Cytochemical differentiation between the pentose and desoxypentose nucleic acids in tissue sections. *Quart J. Mic. Sci.*, **87**, 203.

31. SCHRANK, A. R. 1944. Relation between electrical and curvature responses in the Avena coleoptile to mechanical stimuli. *Plant Physiol.*, **19**, 198-211.

32. ————. 1945a. Changes in the electrical polarity in the Avena coleoptile as an antecedent to hormone action in geotropic response. *Plant Physiol.*, **20**, 133-136.

33. ————. 1945b. Effect of mechanical stimulation on the electrical and curvature responses in the Avena coleoptile. *Plant Physiol.*, **20**, 344-358.

34. ————. 1946a. Electrical and curvature responses of the Avena coleoptile to transversely applied direct current. Pp. 217-231 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

35. ————. 1946b. Analysis of the effects of gravity on the electric correlation field in the coleoptile of Avena satina. Pp. 75-121 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth. University of Texas Press, Austin, 391 pp.

36. ————. 1946c. Note on the effect of unilateral illumination on the transverse electrical polarity in the Avena coleoptile. *Plant Physiol.*, **21**, 362-365.

37. ————. 1946d. The effect of light on the electrical polarity and the rate of elongation of the Avena coleoptile. *Plant Physiol.*, **21**, 465-475.

38. SEIFRIZ, WILLIAM. 1938. The physiology of plants, John Wiley and Sons, Inc., New York.

39. WILKS, S. S., & LUND, E. J. 1946. The electric correlation field and its variation in the coleoptile of Avena satina. Pp. 24-74 in: Lund, E. J. & collaborators, Bioelectric Fields and Growth, University of Texas Press, Austin, 391 pp.

Growth, 1947, **11**, 177-184.

THE EFFECT OF INCREASED NICOTINIC ACID IN THE EGG ON THE DEVELOPMENT OF THE CHICK EMBRYO

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It has been demonstrated by Briggs, Mills, Elvehjem, and Hart (1942) that the chick embryo requires a dietary source of nicotinic acid for optimal growth and for the prevention of "chick blacktongue", an analogue of canine blacktongue and human pellagra. Further, Dann and Handler (1941) have observed that the chick embryo has the capacity to synthesize nicotinic acid and that its content in the hen's egg increases ten-fold during the course of incubation. The latter observations are similar to those of Snell and Quarles (1941), who found that the original content of nicotinic acid increases steadily during incubation to a final value twenty times as high. It is evident from these observations that nicotinic acid is a fundamental component of the embryonic diet of the chick, since it is already present in the un-incubated egg and also since a deficiency of it causes disease and inhibits the growth of the chick.

Since a definite relationship exists between the chick embryo and the amount and nature of its food supply, it was of interest to the writer to study the effect upon the embryo of increasing the nicotinic acid-content of the egg. The method employed has been used repeatedly with other substances. It has been observed in several instances, for example, that the food supply of the embryo can be altered either by the controlled feeding of the hen laying the egg, or by the direct injection of substances into the egg or both. The facility with which this can be done makes possible the utilization of several methods of studying the embryo in relation to its food supply. Thus, Taylor, Thacker, and Pennington (1941) were able to increase the amount of pantothenic acid in the un-incubated egg by injection and by supplementing the diet of the hen with more than the daily requirement.

In the experiments described below, the nicotinic acid available to the chick embryo was increased by the injection of 20 mgm. of the substance into the un-incubated egg to determine the effect, if any, upon the developing embryo.

Methods

Eggs from a flock of selected Plymouth Rock hens were obtained from a nearby farm in Maryland. Before incubation, the blunt end of the egg was washed in Lugol's solution and after drying, a small window 5 mm. square was made in the shell; 2 ml. of albumen were removed with a small graduated pipette and the albumen was replaced with the same amount of 1% (20 mgm.) nicotinic acid in Tyrode's fluid. Care was taken to avoid air bubbles while withdrawing the albumen and during the injection. All instruments were sterilized by washing with 80% alcohol and by burning over a flame.

After injections were made, the shell-membrane and shell were replaced and sealed with warm paraffin. The eggs were then incubated in an Asta electric oven at a temperature of 38-40 degrees centigrade. At the end of incubation periods of 48, 72, and 96 hours, with an allowance of 4 hours in computing temporal ages, the blastoderms were examined with a dissecting microscope and the observations were recorded. The embryos were then removed from the yolk, fixed in Bouin's fluid, stained in borax-carmine, and mounted in clarite for further study. To facilitate this study, the embryos were divided into 3 groups: 48-hours, 72-hours, and 96-hours. Significant conclusions for the 96-hour group are difficult to make because of its limited size.

Description of Experiments

Table I gives a record of all injections made. A total of 217 eggs were injected with 2 ml. of 1% nicotinic acid and 82 were injected with 2 ml. of Tyrode's fluid, the latter serving as controls. Embryos which showed no macroscopic effects and hence are similar to the uninjected controls are classed as normal. All experimental embryos with a pulsating heart are included in the data. The Tyrode fluid-injected controls which showed abnormalities are similar to those already considered by Catizone and Gray (1941) and Gray and Worthing (1941).

In the 48-hour group, embryos which were injected with nicotinic

acid showed a delay in development as well as abnormalities of the nervous and vascular systems. Of the 48-hour controls, however, only 1 showed a delay in development with no apparent abnormalities of the nervous and vascular systems. The 72-hour embryos of the nicotinic acid group showed abnormalities similar to those of the 48-hour group. A relatively small number of the 72-hour controls also showed abnormal development. These, however, are typical of the saline-injected embryos (vide supra). These results suggest that the 2 mgm. of nicotinic acid injected into the egg before in-

TABLE I
RECORD OF INJECTIONS OF 1 PER CENT NICOTINIC ACID

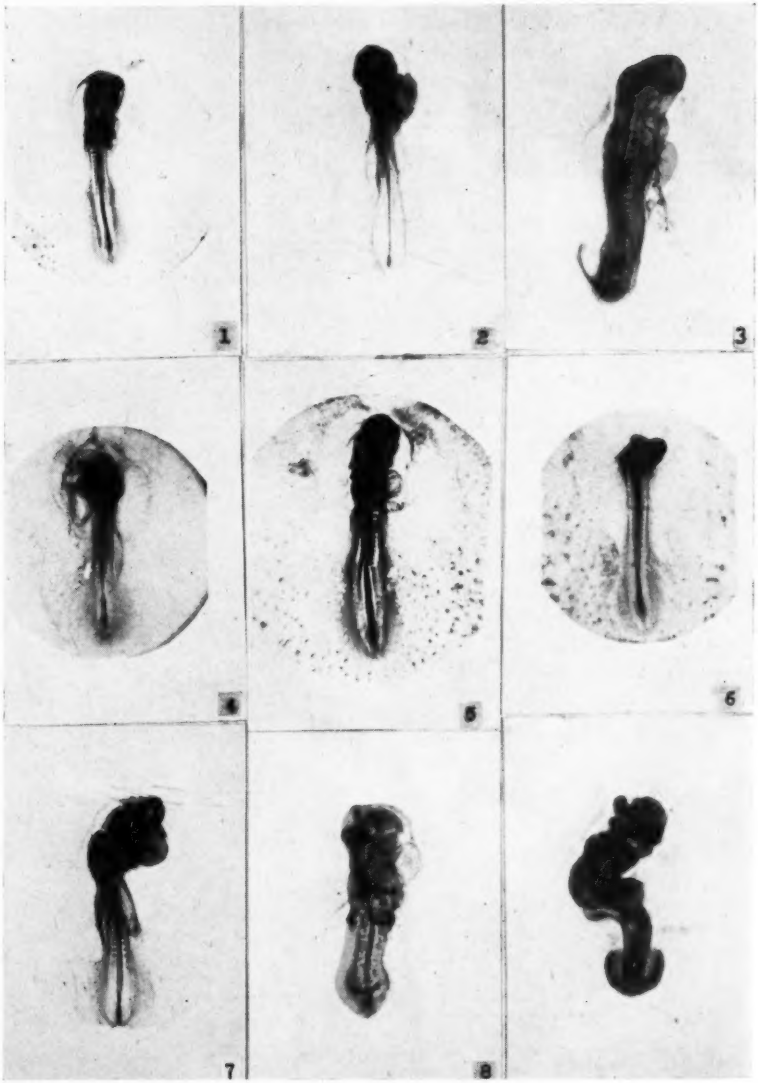
Age at Injection	Age at Fixation	No.	Eggs	Normal	Dead	Retarded Development	Abnormal Central Nerv. Sys.	Abnormal Blood Vessels	Abnormal Heart
0	48 hours	N	63	5	26	32	32	32	32
0		T	21	14	6	1	0	0	0
0	72 hours	N	125	14	49	62	62	62	62
		T	55	35	12	8	8	8	8
0	96 hours	N	29	3	10	16	16	16	16
		T	6	4	2	0	0	0	0

TABLE II
PERCENTAGE COMPARISONS OF INJECTIONS

				%	%	%	%	%	%
0	48 hours	N	63	7.9	41	50	50	50	50
0		T	21	66.0	28	4.7	—	—	—
0	72 hours	N	125	11.2	39	49	49	49	49
0		T	55	63.0	21	14	14	14	14
0	96 hours	N	29	10.0	34	55	55	55	55
		T	6	66.0	33	—	—	—	—

N Nicotine acid—injected
T Tyrode fluid—injected

cubation increase the amount already present to toxic concentrations. This increase in concentration of the acid has an inhibiting effect upon the development of the embryo which results in the abnormal formation of the nervous and vascular organs and probably other structures as well. No attempt is made here to present the data in terms of statistical reliability since there is an obvious difference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EXPLANATION OF FIGURES

Fig. 1. Delayed 48-hour embryo, injected with 2ml. of 1% nicotinic acid, showing absence of vitelline circulation and malformed brain.

Fig. 2. Embryo of 72 hours, injected with 1% nicotinic acid, showing destroyed brain, malformed heart and absence of vitelline blood vessels.

Fig. 3. A 72-hour embryo, injected with 1% nicotinic acid, showing club-shaped optic vesicles, crowded somites, and a tail-like growth from the tail-fold.

Fig. 4. A 72-hour embryo, injected with 1% nicotinic acid, showing exaggerated brain flexures, heart extending in front of the brain, and absence of vitelline blood vessels.

Fig. 5. A delayed 48-hour embryo injected with 1% nicotinic acid.

Fig. 6. A 48-hour embryo, injected with 1% nicotinic acid, showing delayed development, and the heart in front of the much reduced brain.

Fig. 7. A 72-hour embryo, injected with 1% nicotinic acid, showing a malformed brain, and absence of vitelline blood vessels.

Fig. 8. A 96-hour embryo, injected with 1% nicotinic acid, showing crushed brain, malformed heart, and crowded somites.

Fig. 9. A 72-hour embryo, injected with 1% nicotinic acid, showing the "sinuous type" nervous system, exaggerated cervical flexure, and poor vitelline circulation.

in the nature of the effects produced by the two substances injected into the egg.

Effect Upon the Nervous System

Table I shows that of the 63 embryos of the 48-hour group, 32 or 50% showed abnormalities of the nervous system. Typical effects include: a failure of the neural folds to close, thus resembling the "open type" brain described by Catizone and Gray (1941); flattening of the brain vesicles; undifferentiated brain vesicles; persistence of the optic vesicles (failure to form optic cup) so that an elongated club-shaped vesicle is produced; and a multi-segmented or wavy appearance of the brain. Embryos of the last type are similar to "sinuous type" obtained with lead by Catizone and Gray (1941). In many cases the brain was reduced in size, while others showed an abnormally large brain.

In the 72-hour control series, although abnormalities of the brain appear, they are not of the same character of those of the experimental group. In general they include a reduction in the size of the brain and incomplete closure of the neural tube, due probably to a general delay in development. Of the 125 experimental embryos of the 72-hour group, 40% showed defects of the nervous system. These abnormalities are similar to those of the 48-hour group. In both the 48-hour and the 72-hour groups there is a consistent absence of the normal brain flexures for these stages. The whole picture is one that suggests that flexing of the brain has been inhibited. The effect upon the somites seems to be due to the malformation of the nervous system, since embryos which showed less pronounced abnormalities of the nervous system exhibited normal somites.

Effect Upon the Vascular System

The effect upon the vascular system of increasing the nicotinic acid-content of the egg is less pronounced than that upon the nervous system. The abnormalities involve mainly the shape and position of the heart and the degree of differentiation of the vitelline blood vessels. In many embryos, the heart lacked the characteristic curvature of the normal heart. In others, it was a shapeless mass of pulsating tissue which contained very few blood cells. In several cases the heart protruded from the left side of the

embryo or extended in front of the brain or above it (omphalocephaly). The vitelline blood vessels were either poorly developed or entirely lacking. In general, the whole vascular area appeared pale and anemic, due either to a deficiency of erythrocytes or of hemoglobin or both.

Discussion and Conclusion

The injection of 2 ml. of 1% nicotinic acid into the un-incubated egg produces abnormalities in the nervous and vascular systems of the embryo that are not found in the saline-injected controls. Since the use of nicotinic acid in one group alone is the only difference between the two groups, it is assumed that the effects observed in the experimental embryos are due to that difference namely, the nicotinic acid. Apparently the concentration of nicotinic acid used in this work is higher than that which stimulates growth in the embryo. This conclusion is similar to that of Taylor, Thacker, and Pennington (1941), who worked with pantothenic acid. These workers found that low concentrations of pantothenic acid stimulate growth of the nervous system, while higher concentrations inhibit the development of the central nervous system.

The toxic effects of relatively high concentrations of nicotinic acid are manifested in the abnormal development first, of the nervous system and second, of the vascular system, since the effect upon the nervous system is greater than that upon the vascular organs. This observation leads to agreement with Weiss (1939), who has observed that any deleterious agent has its greatest effect upon the region of greatest physiological activity namely, the nervous system. The second effect is upon the vascular system, which is next in physiological activity.

Among the abnormalities of the nervous system which appeared in this work are those which are similar to the "sinuous type" and "open type" central nervous system obtained with lead by Catizone and Gray (1941). From the results obtained with nicotinic acid, however, it is probable that lead is not specific in this respect.

References

BRIGGS, G. M., MILLS, R. C., ELVEHJEM, C. A., & HART, E. B. 1942. Nicotinic acid in chick nutrition. *Proc. Soc. Exp. Biol. and Med.*, **51(1)**, 59-61.

CATIZONE, OLGA, & GRAY, P. 1941. Experiments on chemical interference with the early morphogenesis of the chick. II. The effect of lead on the central nervous system. *Jour Exp. Zool.*, **87(1)**, 71-82.

DANN, W. J., & HANDLER, P. 1941. Synthesis of nicotinic acid by the chick embryo. *Jour. Biol. Chem.*, **140(3)**, 935-36.

GRAY, P., & WORTHING, HELEN. 1941. The effects of tetanus toxin on the morphogenesis of the nervous system. Experiments on chemical interference with the early morphogenesis of the chick. *Jour. Exp. Zool.*, **86(3)**, 423-428.

SNELL, E. E., & QUARLES, E. 1941. The effect of incubation on the vitamin content of the hen's egg. *Jour Nutrition*, **22(5)**, 483-489.

TAYLOR, A., THACKER, J., & PENNINGTON, D. 1941. The effect of increased pantothenic acid in the egg on the development of the chick embryo. *Science*, **94(2449)**, 542-543.

WEISS, PAUL. 1939. Principles of Development. Henry Holt Co., New York.

Growth, 1947, **11**, 185-192.

BOOK REVIEWS

(JESSE P. GREENSTEIN. *Biochemistry of Cancer*. Academic Press Inc., New York, 1947. Pp. 389.)

REVIEWED BY GERRIT TOENNIES

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Dr. Greenstein deserves thanks for having written this book. "With an introduction to current cancer research" could well be a subcaption to its title; and a great need exists for a book of that compass at a time of rapidly expanding activities in the research attack on cancer. "Cancer research is a focus of many independent scientific disciplines, biological, chemical and physical"; and the author has looked into many of them for his own orientation; in this book he shares his experiences, impressions and conclusions with present and future fellow workers.

The first, smaller half of the book contains a survey of the descriptive phases of cancer, histology and pathological terminology, experimentally developed animal strains and cancer types, and further discusses the roles of synthetic chemicals, nutritional factors, hormones and viruses in the genesis and development of cancerous growths. These topics are covered in six chapters comprising about 150 of the 350 textual pages, documented with 590 of the 940 literature citations. Because of the subjects covered and because of the instructive, readable and well documented coverage these chapters are essential. But the essence of the book will be found in chapters VIII and IX (Chemistry of Tumors and Chemistry of the Tumor-bearing Host) with its 180 pages and 350 references. In this (physically and scientifically) weightier part of the book the author speaks with the authority of the geographer describing the territories he has seen himself. It is legitimate that in these chapters 15 per cent of the references deal with the author's own work, compared with less than one per cent in the earlier chapters, and we would have been none the worse if the author had not

yielded to modesty in omitting his own name from the cumulative author index. Or, if this instrument, which is additional to the chapter indices, is for the primary benefit of those of us who in a new book first search for their own names, its space might well be saved to be given over to a greatly enlarged and improved subject index which this reviewer hopes will be a feature of the book's second edition.

The first part of the book requires reading but the second part demands studying. Much of it is occupied with an account of the author's own work in the enzymatic characterization of malignant tissues; an account which is well organized, and at the same time detailed and thoroughly documented, and which does not fail to integrate the author's prodigious efforts with the work of others as shown by the abundant bibliography. If the reader puts down the book with the feeling of having learned much and knowing little it is no discredit to the teller of the story. Whether we agree with him that "biochemistry as a pursuit of professional stature, was added to the oncological sciences only about a decade ago", or whether we grant chemical cancer research the maturity of a quarter of a century (recalling that Warburg's "Metabolism of Tumors" appeared in 1930), we are dealing with a discipline and its disciples both young and growing, perhaps doctrinaire at times but never dogmatic.

Dr. Greenstein rebuilds before our eyes the investigative mosaic, the outlines of which after less than a decade of intensive work led him to conclude that malignant tissues tend to approach a common enzymatic pattern characterized by disappearance or decrease of functional capacities of the normal tissues of origin. However, he is at pains to emphasize that "the experiments described have but scratched the surface of the problems". He is alive to the fact that enzyme deficiencies give no clue to the crucial positive attributes of the cancer cell, viz. invasiveness and autonomy, and that "future research may reveal the presence of enzymes in neoplasms unique to these tissues". Enzymatic deficiencies may be "caused by changes in the protein moiety of these enzymes", there are "possible qualitative differences between the nucleic acids of normal and of tumor tissues", there is the problem of whether "the proteins of a tumor are different from the proteins of the normal tissues of origin", a problem which "up to the present time has received little attention", and there "may

be novel synthetic pathways peculiar to malignant neoplasis". In the stimulating section on "Tumors and the Problem of Synthesis" it is pointed out that nothing is known concerning the nature of the apparent imbalance in cancer between synthesis and lysis of tissue protein. One is tempted here to add to the author's "graveyard" of many a scientific reputation (in the past history of cancer research), and the "veritable wonderland" of much of the older chemical cancer work, the "no-mans-land" of biological protein synthesis, which perhaps in the future will yield to the magic keys of the isotopes.

Over 100 tabulations and nearly half as many diagrams and illustrations help to make the book an extraordinarily concentrated one. This is true despite occasional diffuseness of language and repetitiousness in the exposition of ideas; in which the book is not lacking. The reader interested in facts and thoughts rather than in form may waver but will not balk at linguistic liberties such as: "Simply because certain normal tissues possess properties which in particular instances overlap those of tumors, does not invalidate this conclusion".

The book is pervaded by the warmth of the author's personality and his scientific enthusiasm and energy. But it also vividly reveals his scientific caution in such statements as: "it seems probable from the data available that each cancer does not necessarily represent a separate neoplastic phenomenon, but this may only be an oversimplified chemical view point". Particularly in the introductory and concluding chapters a strong plea is made for cooperation among the different biological disciplines and between the members of the latter and of the medical profession. The book should be rewarding reading for all.

(Edited by M. DEMEREC. *Advances in Genetics.* Volume I. Academic Press Inc., New York, 1947. $7.50. xvi+458 pp.)

REVIEWED BY P. W. WHITING

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Genetics, *sensu stricto,* the study of the laws governing the transmission of hereditary traits, has developed from a science of primary interest in itself into a technique essential to investigations in many diverse fields. Knowledge of the laws of heredity is as much a prerequisite for up-to-date immunology as for a comprehension of many aspects of cell physiology, the chemistry and physics of chromosome structure and function, simple mitosis and the whole chain of meiotic phenomena or, in other words, the science of cytogenetics. The same applies to a host of other sciences including developmental genetics, whether it concerns the shape and size of melons, growth rates in different mutant types of Drosophila or pigment formation such as melanin in the hairs of mammals or anthocyanin in flowers.

The ten review papers bound together in "Advances in Genetics. Volume I" are written by well-known and competent authorities. Each article is quite separate and unrelated to the others. What is presented under "Forage Crops" for example is of no necessary interest to one studying "Immunogenetics". However, much of the detail can be skipped by the non-specialist and the volume as a whole should be useful as a source of general information. For one wishing to become familiar with any one of the specialized subjects, the article concerned will serve as an excellent introduction which should be comprehensible to a non-geneticist, provided he possesses a knowledge of the laws of heredity acquired from attending a course or from independent study of a text. The volume is not intended as a textbook of genetics but should be useful in advanced classes. The adequate bibliographies accompanying each paper should serve to guide the reader intent on delving more deeply into the subject.

"Cytogenetics and Breeding of Forage Crops" (60 pp.) by Sanford S. Atwood deals with highly practical problems of improvement of

grasses and legumes used for pasture and hay. The importance of self-sterility and self-fertility is emphasized; polyploidy, apomixis and polyembryony are discussed as also species hybrids, inbreeding, mass selection and the improvement of strains. "The forage crops, as a group, present an array of complex problems unequalled in most other groups." There are listed 350 references.

"Cytogenetics and Speciation in Crepis" (25 pp.) by Ernest B. Babcock traces the evolutionary history of this monophyletic genus of 196 closely related species. Chromosome groups are shown. The genetic processes of speciation include reciprocal translocations, gene mutations, interspecific hybridization, polyploidy and apomixis in decreasing order of importance. There are listed 39 references.

"Speciation in Fishes" (38 pp.) by Myron Gordon discusses the distribution in time and space of seven dominant multiple alleles in the platyfish *Platypoecilus maculatus*. The patterns determined by these alleles are used as indices in the genetic analysis of natural fish populations of the rivers of Mexico and Guatamala. Factors determining natural isolation from the swordtail *Xiphophorus helleri* are discussed. Intergeneric hybrids are readily obtained under aquarium conditions, although none has ever been taken in the wild. There are listed 39 references.

"Immunogenetics" (27 pp.) by M. R. Irwin embraces genetic characters as yet detectable only by immunological reactions. The well-known antigens of human cells are briefly reviewed including the rhesus factors. A recent review article by Fisher (*The American Scientist*. 35: (1):95-102, 113) is more complete in this respect. Investigations, largely by the author, on antigens in relation to species differences in doves are discussed and the large number, 40, of cellular antigens demonstrated in cattle is noted, making possible over a trillion different possible combinations. Results of experiments on other forms are more briefly considered. The references number 93.

"The Origin and Evolution of Maize" (47 pp.) by Paul C. Mangelsdorf is a comprehensive treatment of the problems involved including morphological and cytogenetic relationships in the Maydeae. Cytogenetics of maize-Tripsacum and of maize-teosinte hybrids are discussed. Reasons are given for rejecting the teosinte hypothesis. Teosinte is probably a hybrid between maize and Tripsacum differing from the former not in a few genes with large effects nor by a

large number of genes scattered at random over the chromosomes, but by a relatively small number of blocks of genes inherited usually as units. For this reason synaptic pairing in hybrids of maize with some varieties of teosinte is good but with other varieties irregularities appear. Evidence is presented bearing upon the pod corn hypothesis. However, "all of these facts combined still do not prove that pod corn is the progenitor of modern maize. Indeed, final proof of this can come only if wild corn is discovered and found to be homozygous for the tunicate character." There are 89 references.

"The Genetics of Cattle" (53 pp.) by Robert R. Shrode and Jay L. Lush supplements rather than replaces earlier views of this very extensive subject. Inheritance of color and of lethal, sublethal and undesirable traits is discussed. Evaluation of breeding worth, beef qualities, milk and butterfat production and many other subjects are treated including artificial insemination. Crosses with other species, inbreeding, crossbreeding and grading are considered. Many differences in physiological traits are discussed. References cited are 237.

"Recent Advances in the Genetics of Paramecium and Euplotes" (96 pp.) by T. M. Sonneborn is concerned with the ciliated Protozoa, the genetics of which "entered a new phase in 1937 with the discovery of mating types in *Paramecium aurelia*. For the first time, it became possible to cross-breed genetically diverse stocks as easily as in higher organisms, and thus pursue genetic studies by means of Mendelian methods." A complete review of the cytology and genetics of *P. aurelia* is given since this species has been thoroughly investigated both by the older and by the newer methods. This then serves as an extensive introduction, after which other species of Paramecium, especially *bursaria,* and of Euplotes are discussed. It is shown how Mendelian methods are applied to this group, in which the nuclear conditions are very different from those of the "higher" forms. The bearing of these studies on the nature and function of the macronucleus is treated at length and cytoplasmic factors, especially kappa, are given much consideration. References number 170.

"Mutations in Wild Populations of Drosophila" (44 pp.) by Warren P. Spencer includes a discussion of the different methods of population analysis (for visibles and for lethals) and also a summary of work done on each species. It is estimated that the actual number of natural and induced mutations found in the genus is probably over

100,000 rather than the 500 to 1,000 stated in many texts. Most of the paper concerns *D. melanogaster* as the bulk of the work has been done on this species. Contradictions in the findings of different investigators are explained in part as resulting from the nature of sampling, but there are real differences in population structure related to environment. This fly is actually an introduced and semi-domestic species in all of the geographical areas where studies have been made. By way of contrast, population work is reported on some of the other Drosophila species, especially *pseudoobscura*. It is pointed out that much progress has been made "since those days not so long ago when many sceptics considered the bottle mutations of the geneticist as interesting phenomena but with little or no bearing on what was occurring to species in the woods, the fields and the mountains." However, in the opinion of the reviewer, it is certain that some and perhaps a majority of the mutant forms used for linkage mappings are "bottle" mutations, not in the sense that they occur only in "bottles", but as having no influence on species in the wild. This fact in no way negatives the significance of mutations in species formation. References cited are 58.

"Types of Polyploids: Their Classification and Significance" (27 pp.) by G. Ledyard Stebbins, Jr. is confined to the flowering plants and "to those aspects in which the relation between natural and artificial polyploidy is of major theoretical and practical importance." The paper presents the basis for the classification of polyploid types with the reasons for divergence in definitions. Differences in genic content and in structural arrangements of chromosomes are not highly correlated and the latter may be of very minute sections. It is therefore difficult and often impossible to consistently separate auto- from allopolyploids. Polyploidy is regarded as a conservative rather than a progressive force, involving "new combinations of characters, rather than the origin of the characters themselves. Such fundamental changes as from polypetaly to sympetaly, from hypogyny to epigyny, and from actinomorphy to zygomorphy have probably been produced by successive genetic changes on the diploid level." There are 102 references.

"Cytogenetics of Gossypium and the Problem of the Origin of New World Cottons" (12 pp.) by S. G. Stephens classifies the species of this morophyletic genus into three groups,—two diploid (13 chromo-

somes) Old World and American, and one amphidiploid (26 chromosomes) New World and Polynesian, on the basis of cytogenetic evidence correlated with geographic distribution. The problem of the origin of the three amphidiploid species is considered; Harland's theory of ancient origin is thought doubtful and difficulties with the theory of recent origin are presented. Cytogenetic studies are regarded as the most fruitful and least speculative line of attack. There are 69 references.

An Author Index and a Subject Index are appended for the entire volume.